Sensory and preference evaluation of the addition of organic acids to white wines

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Abstract

The aim of this work was to evaluate the response of a trained tasting panel to the addition of organic acids in white wine. The tasters were characterised through questionnaires in relation to age, gender, smoking habits, expertise and wine preference (Vinotype). Two taste functions were also determined, concerning 6-propyl-2-thiouracil (PROP) status and saliva flow. Organic acid detection and recognition thresholds were determined by triangular tests. Perithresholds concentrations of lactic acid and lactic acid plus succinic acid were spiked in two white wines of different acidity and preference was evaluated by visual analogue scale (VAS).

The detection and recognition thresholds, expressed in tartaric acid, were of 1.64 g/L and 3.24 g/L for tartaric acid; 1.98 g/L and 3.48 g/L for lactic acid; and 0.88 g/L e 1.05 g/L for succinic acid. The addition of lactic acid (1.92 g/L) and lactic acid (1.92 g/L) plus succinic aci (0.32 g/L) in two white wines did not induce differences ($p<0.05$) in wine preference. However, tendencies were observed in the increase of preference with acidification in the less sour wine and decrease in the sourer samples. Considering all wines and acidified samples, the preference was higher ($p<0.05$) in non-smokers, intermediate expertise, sensitive vinotype, PROP tasters and sweet dislikers. This different appreciation was dependent on wine initial acidity. The sourer wine was preferred by intermediate experts, PROP tasters and sweet dislikers. The less acid wine was preferred by non-smokers, sensitive vinotype and PROP tasters. Overall, the results demonstrated the acidity appreciation was dependent on wine initial acidity together with physiological and individual preferences.

Keywords: Sensorial analysis, wine preference, organic acids, wine acidity

Resumo

O objetivo deste estudo foi avaliar a resposta de um painel treinado de provadores à adição de ácidos orgânicos em vinho branco. Os provadores foram caracterizados através de questionários em relação à idade, sexo, hábitos de fumo, perícia e preferência por tipos de vinhos (“Vinotype”). O painel foi também avaliado em relação à sensibilidade ao gosto amargo, através da prova do composto 6-propil-2-tioracil (PROP) e ao fluxo salivar. Através de testes triangulares foram determinados os limiares de detecção e de reconhecimento de vários ácidos orgânicos. A adição do ácido láctico e do ácido láctico mais ácido succínico, em concentrações próximas dos limiares, permitiu avaliar o seu efeito na preferência em dois vinhos brancos de diferente acidez inicial.

Os limiares de detecção e de reconhecimento, expressos em ácido tartárico, foram de: 1,64 g/L e 3,24 g/L para o ácido tartárico; 1,98 g/L e 3,48 g/L para o ácido láctico; e 0,88 g/L e 1,05 g/L para o ácido succínico, respectivamente. A adição de ácido láctico (1,92 g/L) e de ácido láctico (1,92 /L) mais ácido succínico (0,32 g/L) a dois vinhos brancos não permitiu obter diferenças na preferência ($p<0{,}05$), embora as tendências observadas fossem de aumento da preferência no vinho menos ácido e de diminuição no de maior acidez fixa inicial. Considerando todos os vinhos em conjunto, observou-se que, dentro de cada categoria, a preferência foi mais elevada ($p<0{,}05$) para os não-fumadores, os de conhecimento intermédio, os *sensitive*, os *tasters e* os que não gostam de açucar. Estas diferenças na apreciação variaram, também, em função dos dois vinhos provados. O vinho mais ácido foi preferido pelos de conhecimento intermédio, pelos *tasters* e pelos que não gostam de açúcar. Enquanto, o vinho menos ácido foi preferido pelos não-fumadores, sensitive e pelos tasters. No seu conjunto, os resultados demonstraram que a apreciação da acidez em vinhos brancos depende da sua acidez inicial e das características e preferências de cada indivíduo.

Palavras-chave: Análise sensorial, ácidos orgânicos, acidez, preferências sensoriais.

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TCA – tricarboxylic acid cycle

MLF – Malolactic fermentation

ML– Malolactic

INAO– Institut National des Appellations d'Origine

A– Wine A

AL– Wine A spiked with lactic acid

ALS – Wine A spiked with lactic and succinic acids

B– Wine B

BL– Wine B spiked with lactic acid

BLS –Wine B spiked with lactic and succinic acids.

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Introduction

The culture of wine has spread out through the Middle east, the Nile valley and the Mediterranean basin since the early sign of wine production in the Fertile Crescent area, approximately 6000-8000 years ago. Romans played an important role in the development of wine, in production, storage and classification in Europe, including areas that are major producers such as France, Italy and Spain. Besides romans, religion was algo fundamental in preserving the methods and skills of wine production, especially after the fall of the Roman Empire as monks continued to produce wine in their monasteries (Tchernia, 1983). Thus, production and consumption of wine has accompanied western civilization for thousands of years and it is clear that wine has been playing fundamental economic, social, political and ideological roles in the life of our societies in different parts of the world (Unwin, 2005).

1.1 Sensory analysis

Sensory evaluation is a scientific discipline used to evoke, measure, analyze, and interpret reactions to stimuli perceived through the senses (ASTM, 2005).

Tasting wine has always been a part of the standard operations of a winery. Describing the sensory characteristics of a product has been a common practice in food and beverage industry since a long time ago (Maitre et. al., 2010). The use of sensory evaluation techniques has increased since the late 1980s especially under the leadership of Ann Noble at the University of California, Davis. However, very few wineries were actually using sensory techniques in their winery operations, except in research and development projects and often in collaboration with academic partners. Implement a program of sensory analysis is understood as a costly investment. Sensory analysis has to be seen as a business tool instead of just a research tool (Lesschaeve, 2007).

Pretorius et al., (2006) have clearly enunciated the marketing view of quality by stating "quality is defined as sustainable customer and consumer satisfaction". However, wine experts cannot predict consumer liking scores or market success. Segmentation based on sensory preference is needed for a different approach to wine design style and wine marketing. Understanding the desires of the most of consumers is far more lucrative than a select group of connoisseurs.

Sensory techniques are well documented and accessible in order to better characterize wine sensory properties. Nevertheless, there is not enough available and trained experts in companies, so the positions tend to be taken by people who have received little or no training in sensory analysis. Sensory evaluation is taught worldwide in most enology and viticulture programs at the university level; however, students seldom apply in their working practices what they have learned in their sensory course(s). Moreover, certain programs labeled as “wine sensory evaluation courses” in fact teach “wine appreciation” and not the sensory techniques associated with good practices of sensory evaluation (Lawless & Heymann 1998).

1.2 Consumer studies

Wineries have increasingly been interested in the potential of combining sensory evaluation and market research to enhance their understanding and targeting of consumer wine preferences. Wine companies have recognized the importance of better understanding consumer preferences in order to sustain and develop their business in the competitive global marketplace. Therefore, this allows wineries to develop their wine styles according to the needs, desires and expectations of the consumer (Lesschaeve, 2007). Many studies have been conducted in order to understand what are the sensory attributes that lead to acceptance of the consumer wines.

For example, Australian wine industry believes that the descriptor "green" associated with methoxypyrazines is undesirable and, therefore, seeks to minimize its impact. However, King et al.,(2011) suggest that a sizeable proportion liked the “green” styles. This leads us to believe that it is an opportunity for Australian producers to increase methoxipyrazines levels and, consequently, the green characteristics in their wines, in conjunction with “tropical fruit” aromas. Williamson et al.,(2012), for red wines, concluded that most of the Chinese population prefer wines with the attributes “red berry”, “floral/confectionary” and “vanilla” aroma or flavor and also present high fruit aftertaste and sweetness. In the same study, Australian and Chinese prefer lighter wine red berryflavor with low level of acidity and bitterness. While 23% of Australians associate sweetness as a negative feature, the Chinese do not see as such.

1.2.1. Consumer segmentation

The understanding of individual differences in orosensation is of great interest for the wine industry, these differences may represent opportunities for developing new products based on the different responses of individuals. A large number of factors contribute to the differences noted in the perception of taste or non-taste oral sensations, including gender, age, ethnicity, salivary composition and salivary flow rate (SFR), experience and environment. However, the most important factor in perception of oral stimuli is genetic variation (Pickering et al., 2008).

1.2.1.1 PROP status

The genetic variation is one of the most decisive factors in the individual differences in orosensation and perhaps the greatest influence in relation to food and beverage preferences. This genetic variation in taste is usually evaluated by response to PROP (6-n-propylthiouracil). Many studies show that crystals of 6-n-propylthiouracil (PROP) tasted bitter to some persons but were tasteless too others (Blakeslee, 1931; Snyder, 1931; Fox, 1932). People are divided into supertasters (those for whom PROP elicits no or slight bitterness), tasters (those for whom PROP is mildly bitter) and nontasters (those or whom PROP is intensely bitter). This line of research was spurred by the argument that PROP-tasting genes in humans owed their continued existence to the evolutionary advantage conferred by the ability to reject and avoid bitter toxins (Boyd, 1950). Some studies (Hayes et., al, 2008; Drewnowski, 1991) indicate that supertasters had more fungiform papillae, more taste buds, and a higher density of buds per papilla than did either regular tasters or nontasters. However, Tepper et al., (2001) said that distributions of papillae densities for the three taster groups greatly overlap. Until today there seems to be no evidence that fungiform papillae density differs between subgroups (Garneau et al., 2014, Fisher, 2013). Also, several recent studies show that those who experience PROP as being intensely bitter not only experience heightened overall oral sensation, but also may be more acute tasters, i.e. are able to discriminate small differences between oral stimuli (Prescott et al., 2004; Lee et al., 2008; Hayes et al., 2010).

1.2.1.2 Thermal taste

PROP tasting is not the only responsible for the individual difference in oral sensation. Recently, Green and other coworkers have identified a new marker of individual variation in oral sensation: thermal taste. This phantom-taste is perceived when a small area of tongue is heated and/or cooled. The specific sensations elicited include the basic flavours like a sourness, sweetness, saltiness, bitterness and such as metallic, and vary with the area stimulated and the temperature regime used (i.e. heating or cooling). TRP superfamily cation channel plays a role in thermal taste such as in the transduction of unami, sweet and bitter tastes (Talavera et al., 2005).

According to Pickering et al. (2016) the phenomena of thermal taste and PROP tasting and FP density are genetically and mechanistically independent. In contrast to the PROP responsiveness, thermal tasting has been much less explored in the literature. It is known that thermal tasters (TTS) correspond to 20-50% of the population, these individuals tend to rate both prototypical tastes and most chemesthetic sensations at supra-threshold levels more intense than thermal non-tasters (TnTS). The ability to perceive thermal taste on the tongue tip is positively correlated with the responsiveness to chemical taste stimuli of all kinds throughout the mouth. Individuals differ in the ability to perceive the flavor and that these differences may arise in part from variation in the sensitivity or ‘gain’ of central nervous system processes that are involved in perception of the chemosensory attributes of food (Green & George, 2004).

1.2.1.3 Sweetness preference

The preference for sweetness, rather than bitterness or acidity, is innate and strongly linked to the fact that sugar is the source of energy. As sweetness levels increase, a pattern of hedonic responses that follows an inverted-U has been reported regardless of culture (Prescott, 1998; Prescott et al., 1992, 1997). Linking for sweetness increases with the concentration of sucrose until approximately 10-12% w/v and then undergoes a gradual decrease from this point (Yeomans et al., 2007). However, despite this general and universal approach of sweet tastes, it is known that there are individual variations in response to increasing levels of sweetness. Similarly, Moskowitz (1971) reported that the pleasantness of sugars was not constant over concentration and he later (Moskowitz, et al., 1985) segmented subjects into different clusters by hedonic responses of sweetness. With subsequent studies were obtained hedonic responses to the sweetness in sweet likers and dislikers. Sweet likers (SL) are generally those that show increases in monotone like throughout the range of concentrations studied sweetener, while sweet dislikers (SD) are those that show a monotonic decreasing response or reaches a hedonic asymptote in low or moderate concentrations, followed by a decrease in liking (Kim et. al., 2014). There is evidence that greater liking for sweetness is linked to higher consumption of both added sugars and sweet foods (Duffy et al., 2003; Holt et al., 2000).

According to Sena-Esteves (2016) the sugar preference in wine is correlated with the experience of wine consumption. Novice consumers showed a clear preference for sweetness in wine, while the experienced consumers group did not show any preference for sweet at any concentration.

1.2.1.4 Vinotype

Vinotype is a tool created in 2011 based on the individual's preferences and asses the sensitivity to sugar (Hanni, 2012). There are three questions involved that estimate some elements that the individual values concerning wine. The possible results are: Sweet, Hypersensitive, Sensitive and Tolerant. This test has no scientific basis, as far as we are aware, but it is only a tool for consumers to learn more about their preferences, yet it helps us to understand the sensitivity (https://www.myvinotype.com).

1.2.1.5 Saliva flow

Saliva, the first physiological secretion induced by ingestion of foods or beverages or by oral manipulation, plays an extensive role in the oral cavity and in taste perception. Stimulation by 2% citric acid enhanced the average salivary flow by a factor of 10 (Benedek-Spat, 1973), but individual salivary responses varied from a 50% to a 2500% increase upon stimulation (Jenkins, 1978). Individuals with high-flow (HF) rates responded with higher flows to all stimuli than did low-flow (LF) Individuals, although the relative responses to different stimuli showed proportional sialogic effects of the stimuli among flow groups.

Acids have been reported to appear more sour to Individuals who have lower saliva flow rates, and corresponding lower salivary pH (Cragg, 1937). In contrast, Norris et al. (1984) observed significantly higher sourness ratings among HF Individuals than LF Individuals. The LF Individuals however, were better able to discriminate the sourness differences. It was proposed that due to the lower salivary buffer capacity in LF Individuals, the sourness differences among the acids persisted longer and permitted a better discrimination. However, Bajec & Pickering (2008) suggests that SFR is not associate with the perceived intensity of any oral stimuli instead of previous studies that have reported that either low-flow or high-flow groups rate the astringency of polyphenols higher.

1.2.1.6 Age and Gender

Age has a deteriorating effect on taste sensitivity. Cooper et al. (1959) found that taste sensitivity remains unimpaired until the late fifties, after which it shows a sharp decline. This finding might be influenced by an increase in drug consumption with age. Over 250 commonly used drugs have been reported clinically to affect the sense of taste. However, the decrease in sensitivity with age was generic in nature, even though the extent of the decrease differed for the basic taste qualities and, to a lesser degree, for the compounds within a basic taste. Glanville et al., (1964) found that both males and females showed a gradual increase in sensitivity up to the age of 16–20 years, followed by an exponential decline. The salty and umami taste qualities seemed to be most affected. The elderly have a less specified taste acuity than the young, for which the noise hypothesis provides an explanation, either at a neural level, at a psychological level, or at both levels. Although it seems that renewal and redundancy in the taste system preserve gustatory function in old age (Miller and Bartoshuk, 1991), it is not clear that the functioning of aged taste buds is not impaired. It is known that when the olfactory input is blocked about 70 % of the age differences in taste perception disappeared (Mojet, J., 2004). Age was a significant source of variation in linking scores for the majority of wine styles. According to Pickering & Hayes (2017) this happens in part due to the experience that older consumers can have because they had more time and opportunity to try out different styles of wine. In the same study, young people show a clear preference for sweeter wines while the older consumers prefer drier wines.

1.2.1.7 Culture and ethnicity background

An innate ability to discriminate various tastants is present independent of ethnic background (Holt et al., 2000). Preferences for basic tastes (particularly sweet and bitter) might be determined genetically, or at least be present before birth. However, location or geographical factor can affect individual/group's taste perception. Exposure, experience and food habit (caused by surrounding and culture) may give more significant impact to our taste perception (Baharuddin et al., 2015). This ethnic dietary habits can modify innate preferences as with chilli peppers. Children before the age of 8 years old reject this food, however from this age is deprecated (Rozin, 1996). Some authors reported in fact that cultural practices common in Africa, such as feeding sugar water to infants, resulted in an increased sweet preferences during the second year of life of the child (Beauchamp & Moran, 1982). This indicates that traditions, experience and historical background could have a significant impact on food preferences from the first period of life (Kobayashi & Kennedy, 2002; Mennella, 2014). However, Drewnowski, (2003) recognizes that economic factors can have an impact on food choices, which is the case for sugar and fats because they are foods that feature low price and more palatable. Jamel et al. (1996) found that people who are living in urban areas with high sugar intake preferred sweeter tea compared to people who are living in rural area. Holt et al. (2000) reported that Malaysians' preference of sweeter food compared to Australians is related to higher sugar intake among Malaysians. Holt et al. (2000) confirmed that people with more exposure and regularly consumed sweet food tend to have high preference for sweet taste. However, Prescott et al. (1997) found that there were no differences in the sweet and salty tastes between Australian and Japanese Individuals. The author claim that sweetness and saltiness occurred despite each cultural group being unfamiliar with the sensory characteristics of different subsets of the products drawn from the other culture. The Japanese Individuals in comparison with North americans demonstrate a greater ability to discriminate different intensities of sucrose and of monosodium glutamate, but no differences in ability to discriminate NaCI intensities. Laing et al. (1994) also suggested that differences in responses of Individuals from different cultures to chemosensory stimuli are restricted to reference behavior arising from experience rather than from genetically-based influences. Differences in taste sensitivity to the bitter compound 6-n-propylthiouracil (PROP), which are believed to be inherited has been found to vary between races, Asian, South American-Indian, and African populations have been found to have a higher proportion of PROP tasters than Caucasian (Holt et al., 2000). Despite the innate preference for certain tastes and foods, genetically determined, also observed some ethnic differences

1.3 Sour taste in wines

One of the most important characteristics in wine and musts is related to its acids constitution. Acidity, or sourness, contributes in many direct and indirect ways to the quality of wine. Acidity determines some of the principal factors of the physicochemical equilibrium that affects technological processes, influences microbial activity and wine aging (Curvelo-Garcia & Barros, 2015). Acidity influences the solubility of proteins, polyssaccharides and potassium bitartrate, wine colour and the effectiveness of sulphur dioxide, fining agents and pectin enzymes. Adequate acidity gives freshness, and is regarded to balance any residual sugar and the aroma of wines. Acids determines the organoleptic characteristics, it is common to describe wine without acidity as dull, flat, insipid (Fischer, 2001).

Wine' acid properties are a result of the presence of organic acids or fixed acids (Boulton et al., 1998). Acids come from the grapes, from the activity of the most diverse microorganisms (yeasts, lactic bacteria, acetic bacteria), and from natural chemical processes that occur during the evolution of musts and from different technological processes, namely the acidity correction through the addition of acidifiers. Like the origin, the composition of acids of the musts and wines is very diversified, the contents can vary from the order of a few g/L to concentrations below 1 mg/L, the acid strength and chemical nature are also very different and such as their organoleptic characteristics (Curvelo-Garcia & Barros, 2015).

1.3.1 Organic Acids

Organic acids belong to the most important components that complete the overall character and taste of wine (Zeravik et. al., 2016).The organic acids (non-volatile) give the wines their acidic properties. The main organic acids present in wine are L(+)-Tartaric Acid, L(-)-Malic Acid, Citric Acid, Succinic Acid, L(+) Lactic Acid and D (-)- Lactic Acid (Curvelo-Garcia & Barros, 2015). Ripe grapes contain majour amounts of tartaric and malic acids, although citric acid is algo present at lower doses. In addition, wines contain products of yeast and malolactic fermentation such as acetic, lactic and succinic acids. The content of organic acids in wine depends on the region and its climate during growth and harvest. Wines from warmer regions generally contain more tartaric acid, while wines from colder regions contain more malic acid. Besides the acids already mentioned, there is another organic acid, the gluconic acid that occurs especially in grapes attacked by *Botrytis cinerea*, it is know that this acid is an indicator of the sanity of the grapes, since the gray rot affects greatly the qualities of the future wine .

1.3.1.1 Grape acids

The main organic acids in grapes are L-tartaric acid and L-malic acid (Boulton et al., 1998a). Together, these two organic acids represent up to 90 % of grape juice's total acid content (Jackson, 1994). The third most important organic acid in grapes is L-citric acid.

1.3.1.1.1 Tartaric Acid

Tartaric acid is the main and the strongest acid, being unique since it only occurs in grapes. It is a diprotic (two H+ ions) acid, accounts for a large proportion of a wines acidity. Tartaric acid is derived via a complex transformation from vitamin C (ascorbic acid). This appears to involve L-idonic acid, as a rate limiting step (DeBolt et al., 2006). Normally exists at a concentration between 5-10 g/L in grapes (Boulton, 2013) and actively contributes to the values of fixed acidity and pH. Among the major acids, this is the most resistant to bacterial action, although volta disease may occur, where the total tartaric acid is decomposed. However, nowadays this phenomenon is uncommon. Thus, the tartaric acid is the most used acid for acidity correction (Margalit, 2012) and presents “pure acid taste” (Curvelo-Garcia & Barros, 2015). During maturation of the grapes either by phenomena of combustion or dilution, its content decreases. During alcoholic fermentation, the concentration decreases due to the precipitation of calcium and potassium salts. After vinification, the decrease continues due to the unsaturation of these salts (Margalit, 2012).

1.3.1.1.2 Malic Acid

Malic acid is a diprotic (two H+ions) acid. Is the most widespread fruit acid and, is generally present in grapes at concentrations in the range 2 to 4 g/L. This acid is an important intermediate in the TCA cycle. As such, it can be variously synthesized from sugars (via glycolysis and the TCA cycle), or via carbon dioxide fixation from phosphoenolpyruvate (PEP). Malic acid also can be readily respired, or decarboxylated to PEP via oxaloacetate in the gluconeogenesis of sugars. Not surprisingly, the malic acid content of berries changes more rapidly and strikingly than that of tartaric acid. Organoleptically, it has a "pure and green acid taste". The malic acid may constitute about half of the total acidity of grapes and wine. Its concentration in the fruit tends to decrease as grapes mature, especially during hot periods at the end of the season (Boulton et. al., 2013). This can lead to the production of wine that has a flat taste and that is susceptible to microbial spoilage. Conversely, under cool conditions, malic acid levels may remain high and give the resultant wine a sour taste (Jackson, 2014).

1.3.1.1.3 Citric acid

Citric acid is triprotic acid (tree H+ions), common in plant kingdom and relevant for Enology. Citric acid is in grapes with high concentrations, until 500 mg/L. It is very important in the biochemical and metabolic pathways (Krebs cycle) (Ribérau-Gayon et. al., 2006). This acid presents flavor acid pure and freshness. Another interesting property is the ability to form a complex with Fe being a ferric casse blocking agent. (Curvelo-Garcia & Barros, 2015) Despite improving the acidity taste of wines, their oenological use is limited to 1 g-/- L by the OIV, because it can be degraded by lactic acid bacteria leading to an undesired increase in volatile acidity.

1.3.1.2 Organic acids formed during fermentation

1.3.1.2.1 Lactic Acid

Lactic acid is monoprotic acid. A small amount of lactic acid is produced by yeast cells during fermentation. However, when lactic acid occurs as a major constituent in wine, it derives from bacterial action. The most commonly bacteria involved are lactic acid bacteria. These bacteria produce an enzyme that decarboxylates malic acid directly to lactic acid by process named malolactic fermentation, which is commonly encouraged in red and in some white wines. The major benefit of malolactic fermentation is the conversion of the harsher-tasting, dicarboxylic, malic acid to the smoother-tasting, monocarboxylic, lactic acid (Jackson, 2014). The concentrations of this acid in wines can range from 0 to 2,5 g/L.

1.3.1.2.2 Succinic Acid

In most wines, the presence of succinic acid (diprotic acid) occurs as a consequence of yeast fermentation, rather than being a by-product of grape metabolism (Jackson, 2014). Most of the succinic acid that is excreted by fermenting yeasts is produced during the beginning of alcoholic fermentation, when there is still only 4-5 % (v/v) alcohol (Thoukis et al., 1965).

Succinic acid is one of the commonest by-products of yeast metabolism and therefore occurs in all alcoholic beverages (Margalit, 2012) although its importance in wine is often overlooked because of its weak acidity. Succinic acid can be produced biochemically via the oxidative branch of the Krebs cycle due to mutation of succinate dehydrogenase or via the glyoxylate cycle or even be chemically produced by the decarboxylation of alfa-ketogluraric acid under the action of oxidizing agents, such as hydrogen peroxide (De Klerk, 2010). It is resistant to microbial attack under anaerobic conditions and it is particularly stable in wine and does not change with aging (Margalit, 2012). It is found in wines between 0.5 -1.5 g/L, with red wines having the highest concentrations (mean value 1.0 g/ L) and in white wines lower values (mean 0.7 g / L). Its titratable acidity is 30% higher than tartaric acid (because its molecular weight is 118 g/mol compared to that of tartaric acid which is 150 g/mol). This means that 1.0 g/L of succinic acid produced during the fermentation will add 1.3 g / L of titratable acidity (De Klerk, 2010). However, large quantities of succinic acid will have little effect on wine's pH (Margalit, 2012).

Besides acid taste, succinic acid has a bitter and salty taste (Curvelo-Garcia & Barros, 2015). This bitter-salty taste of this acid limits its use for wine acidification (Jackson, 2014). Some studies indicate that tasters found succinic acid's taste unpleasant compared to tartaric acid's taste and indicated that succinic acid's unusual taste lingered some time after expectoration (Coulter el al., 2004). The taste threshold of succinic acid dissolved in water ranges from 34-35 mg/L (Berg et al., 1955; Amerine et al., 1959), however wines with very high levels of succinic acid were not identifiably salty or bitter according to the results of informal tastings. Succinic increases the sour taste of wine by increasing its buffering capacity (titrable acidity). The buffering capacity of wine is defined as its ability to resist to pH changes, when its diluted with water or titrated with small amounts of base or acid. When wine is tasted, its acids are partially neutralized by saliva in mouth (Boulton el al., 1998a), which explains why the duration and intensity of wine's sourness is related to its buffering capacity.

Considerable amounts of succinic acid can be found in unripe *Vitis rotundifolia* (muscadine) grapes and grapes infected by moulds. However, grape juice from healthy *Vitis vinifera* grapes contains only contains traces of succinic acid. It is the second most significant organic acid in 'Noble' muscadine wines (*V. rotundifolia*) (Lamikanra, 1997). *Botrytis cinerea* and other moulds produce succinic acid from the sugar in grapes (Rankine, 1986). In the botrytised grape juice was found as maximum 783 mg/L of succinic acid.

Succinic acid production depend on different factors, such as type of yeast used, as weel as fermentation conditions, pH, temperature and sugar have a great influence on the production of succinic acid. Since Pasteur (1860) a direct relation between the amount of sugar fermented and the amount of succinic acid formed is known. Succinic acid production by yeasts increases with the increasing fermentable sugar concentrations up to 220 g/L (Shimazu &Watanabe, 1981), but starts to decline at higher sugar concentrations due to an increase in acetic acid production (Caradi, 2003). In general, higher temperatures and higher pH benefit the production of succinic acid. However, with temperatures greater than 30 º C there is a rapid decline in the amount of succinic acid produced by fermenting mesopHilic wine yeasts. Succinic acid production is influenced by the metabolizable portion of the fermentation medium's yeast assimilable nitrogen (YAN) content, which was subsequently termed metabolically available nitrogen (MAN). Ymn is defined as the total amount of nitrogen that can be converted into ammonium ions when sources thereof are metabolized by yeasts. Moderate concentrations of YMN (300 ± 50 mg/L) was optimal for succinic acid production in syntetic grape juice with 200 mg/L fermentable sugar (De Klerk, 2010).

1.3.1.2.3 Acetic Acid

Acetic acid is the main volatile acid in wine, which may also contain other volatile acids such as formic, propionic and butyric. Acetic acid is formed as a by-product during yeast fermentation result of a side reaction of acetaldehyde oxidation. The range of formation is about 200-500 mg/L (Margalit, 2012). At this concentration level it is not noticeable on the palate and has no effect on wine quality, adding complexity to taste and odor (Jackson, 2014). Acetic acid is also formed by acetobacter bacteria in aerobic conditions. The bacteria oxidize the ethanol to acetic acid at concentrations which depend on the air exposure and time. Above 0,8-1,0 g/L, volatile acidity is noticeable and it depreciates wine quality (Margalit, 2012).

1.3 Acidulants

If wines are too low acidity, or possess an undesirably high pH, usually in warm regions, various acidulants can be added. The most commonly used acidulants in grape wine production are tartaric, malic, and citric acids.

Many winemakers prefer to make necessary acid additions to the must rather than solely to the wine. Additions at this stage may help to maintain a low pH during fermentation, enhance color extraction, in the case of red wines, produce more desirable product (Zoecklein, 2012).

As an acidulant, tartaric acid has several distinct advantages. These include its fresh crisp taste, high microbial stability and a dissociation constant (Ka), which allows it to markedly reduce the pH. The main disadvantage of tartaric acid addition is its cost, especially when added to wines high in potassium content. Crystal formation results in most of the tartaric acid being lost due to precipitation. The addition of citric acid avoids these problems and can assist in preventing ferric casse, via its chelating action. Nevertheless, the ease with which citric acid is metabolized by many microbes, meanings that it is microbiologically unstable. Addition of citric acid to must be avoided. When added to grape wine in large quantities, it can result in what many would regard as citric-like flavor. The OIV places a maximum limit for citric acid at 1,0 g/L(Zoecklein, 2012). Malic acid can successfully be used as an acidulant. It will not precipitate like tartaric acid, and the only problem is to prevent it from undergoing ML fermentation. If this is the case in a specific wine which has to be acidified, it is a good choise. If added as racemic acid and MLF is carried on, then the D-malic acid will remain (Margalit, 2012).

Lactic acid is the weakest acid of those permitted to be added, so the addition rate needs to higher to achieve the same pH decrease as malic or tartaric acids. However, the pH decrease is more predictable in comparison with tartaric acid. There are three advantages claimed for lactic acid addition: it can be added just prior bottling without cause for precipitation , it produces a rounder and smoother mouthfeel than malic acid and finally, lactic acid addition increases the formation of lactic esters that contribute to a wine's aroma Another possibility is addition of fumaric acid. This acid can be used for two purposes: malolactic fermentation (MLF) inhibitor, in concentrations higher than 500 mg / L and acidification. If the pH is higher, higher concentrations of fumaric acid are needed. It should be noted that the addition of fumaric acid should not be done before alcoholic fermentation (AF) because the fumaric acid will be degraded by yeast (Ough, 1999). Therefore, if necessary, it should be added only after the racking. Fumaric acid does not exhibit any sensory negative effect when added up to the 1.5 g / L range (Margalit, 2012).

1.5 Other acids used as oenological products

1.5.1 Sorbic Acid

Sorbic acid is usually used as a fungistat rather than as an acidulant or bacteriostat. It can produce unpleasant “geranium” odors when acted on by lactic acid bacteria during malolactic fermentation or afterwards. (Jackson, 2014).It has found use in sweet wines to prevent refermentation, and is used in conjunction with a bacteriocide or bacteriostat like sulfur dioxide.

1.5.2 Ascorbic acid

Ascorbic acid is the natural product more effective in prevention of color and aroma oxidation. Is commonly used in conjunction with potassium metabisulphite (SO2), limiting the oxidations catalyzed by the enzymes lacase and tyrosinase. The use of ascorbic acid should not be added at bottling. The OIV establishes 10 g-/-hl as maximum permited at bottling (Zoecklein, 2012).

The different acids present in grapes and wines are in table 1.1 and organic acids that can beaded to must/wine are in table 1.2.

Table 1.1 Main organic acids present in grape and wines.

Acid	Origin			**Significance**
	Grape	Fermentation	Other	
L(+) Tartaric	+	-		Acidifier
DL- Malic	+	-		Acidifier
Citric	+	-		Acidifier
L(+)- Lactic	-	+	Lactic fermentation of hexoses	Acidifier
D(-)- Lactic	-	+		Acidifier
Succinic	Residual	AF		Acidifier
Acetic	+	AF FML	Lactic fermentation of pentose; Decomposition of citric acid (bacteria)	Grey rot and sour rot indicator
Gluconnic	+	-		Noble rot or gray rot indicator

Abbreviation: AF, Alcoholic fermentation; FML, Malolactic fermentation

Table 1.2 Organic acids that can be added to must/wine.

Acid	Enological product	Residue in wine	Significance
L(+)-Tartaric	Can be only be added to musts under condition that the initial acidity content is not raised by more than 54 meq/l (i.e. 4 g/l expressed in tartaric acid)		Acidifier
DL- Malic			Acidifier
Citric		1 g/L	Acidifier
L(+)- Lactic			Acidifier
D(-)- Lactic			Acidifier
L-Ascorbic	Until 250 mg/L	300 mg/L	Anti-oxidant
Fumaric	No permitted in OIV		Acidifier
Sorbic	Until 200 mg/L		Preservative

Abbreviation: OIV, International Organization of Vine and Wine

1.6 pH

The concept of pH is defined mathematically as log 10 of the concentration of hydroxonium ions in an electrically conductive solution, such as must or wine:

$$PH = -\log_{10}[H_3O^+]$$

The pH value is an equilibrium measure of hydrogen ion concentration or activity and is affected by the degree of which acids in a solution are neutralized. It is easily measured using a pH meter and an electrode. The pH value of a must depends on many factors including the degree of maturity at harvest, the cultivar, the crop level, the season, the soil moisture and the mineral composition available to the vine. Values can range from 2.8 to 3.0 in early-maturity fruit to be harvest for sparkling wine or base wine for distillation, to a desirable range of 3.0 to 3.3 for table wines. Fruit in which the exchange reactions have been more extensive can have pH values between 3.5 and 4.0 and even higher pH values are sometimes observed in extreme conditions, particularly in overripe grapes or in regions with an extended growing season due to cool conditions and an absence of early rainfall. Wines vary considerably in pH, with values below 3.1 being perceived as sour, and those above 3.7 being considered flat. White wines are commonly preferred at the lower end of the pH range, whereas red wines are frequently favored in the midrange. Relatively low pH values in wine are preferred for many reasons. They give wines their fresh taste, improve microbial stability, reduce browning, diminish the need for SO2, and enhance the production and stability of fruit esters (Jackson, 2014).

1.7 The sense of taste

In theory, the term taste should be restricted to five particular qualities: sweetness, sourness, bitterness, saltiness and unami (Reynolds, 2010).The basic tastes serve an important dietary function. It is thought that taste is primarily used to evaluate the nutritious content of food and drink and to prevent the ingestion of toxic compounds. These sensations are mediated by specialised neuroepithelial cells (taste receptor cells), clustered into onion-shaped organs (taste buds), which specifically detect the dissolved substances that come in contact with them (Kinnamon & Margolskee, 1996).

The stimulus hits the sense organ and is converted to a nerve signal which travels to the brain, where information is processed, thus resulting in the perception of taste. The distribution of the four taste receptors on the surface of the tongue is not homogeneous, associating the apical region with the sweet, the lateral ones with the salty and acid and the posterior with the bitter. At present, it is known that all taste buds, contrary to what was previously thought, have a certain degree of sensitivity for each of the primary flavors.

Nevertheless, the term taste, as well as the term flavor, is often used more broadly to designate taste together with other sensations: on hand, retro-nasal perception of aroma, involving interactions of volatile compounds with olfactory receptors situated in the nose cavity; on the other hand, tactile sensations felt in the mouth such as heat or astringency, which correspond to mouthfeel or texture (Reynolds, 2010)

Tastes cells detect sugars and amino acids at very high concentrations, presumably in order to allow us to detect food of high nutritional value. Sweet taste helps to identify energy rich nutrients and it enhances our enjoyment of food, unami helps to identify amino acids, salt helps the intake of required minerals, whilst sour and bitter taste perception helps us the intake of potentially dangerous compounds (Jokie & Clarke, 2011).

1.7.1 Sour taste

Acidity has an unpleasant and aggressive sensation that sharply provokes the palate evoking a mouth-watering effect. Just the thought to biting into a fresh lemon can elicit this reaction. The sharp, assertive, prickly feel of acidity focuses itself at the sides of the tongue and the angles of the jaw. This sensation becomes even more pronounced if we extract the actual flavor of the lemon. With flavor stripped away, only sharp, harsh acidity remains to nettle the palate. It is only when we place acidity in the context of the other elements of wine, that we can appreciate its essential nature (Fischer, 2001). The main function of the acidity in wine is to support the flavorful extracts, indeed, the vitality of the flavors in wine depends on acidity. Acids make wine refreshing and exciting and give it life, enthusiasm, and vigor (Fischer, 2001).

The chemistry of sour taste appears to be simple because it has been only associated with acids. In 1898, sour taste was first linked to hydrogen ions (Richards, 1898).Later, in 1920 it was discovered that acid taste cannot be explained solely by the H+. Organic acids could also stimulate a sour taste response. Organic acids have one or more carboxyl groups. These have H^+ that can be dissociated depending on the strength of the acid. In 2005, it was proposed a new hypothesis for the chemical basis of sour taste of organic acids. According to Johanningsmeir et al. (2005), sour taste intensity is a linear function of the total molar concentration of all organic acid species that have one or more protonated carboxyl groups plus the concentrations of free hydrogen ions. Understanding the perception of sour taste has received less attention than sweetness and bitterness, particularly for mammals (Stewart et al., 1997). Studies indicate that the sour tastants are generally ionic (H+) and act through ion channels on the cell membrane of taste receptors, using a different mechanism from the others tastes.

1.7.2 Sensory thresholds

Thresholds are often considered as the intensity of the stimulus that sets the limit of sensitivity of the sensory system. It is considered that the stimulus intensity below this level has no effect on the sensory system and, therefore, cannot be perceived. Thus, threshold is the absolute value which the sensivity of the sensory system does not allow for detection. In other words, the threshold is considered top a transition point between the existence of sensation and absence of sensation (Bi & Ennis, 1998). There are different thresholds:absolute/detection threshold, recognition threshold, difference threshold and the terminal threshold. The detection threshold is the lowest stimulus capable of producing a sensation. The recognition threshold is the level of a stimulus at which the specific stimulus can be recognized and identified. The difference threshold is the extent of change in the stimulus necessary to produce a noticeable difference. The last one, terminal threshold is that magnitude of a stimulus above which there is no increase in the perceived intensity of the appropriate quality for that stimulus; above this level, pain often occurs (Meilgaard et al., 2007).

Individual sensivity and population sensivity are of interest in sensory analysis. The definition of the population threshold is based on other threshold information (individual thresholds). Thus, the calculation procedure for a population threshold includes: firstly, estimating the individual thresholds (best estimate threshold,BET) and then estimating the population threshold (group BET). The BET is the geometric mean of the highest concentration missed and the next higher concentration when this was followed by at least two further correct responses. Their BETs were determined by calculating the geometric mean of the lowest correct concentration and the next hypothetical lower concentration in the series that would have been presented (Yu & Pickering, 2008).The group BET is the geometric mean of the individual BETs. The graphical solution was also used to evaluate the group detection and recognition threshold.

1.8 Background and objectives of the study

The acidity, as already mentioned, is one of the fundamental characteristics in the evolution and quality of the wine.As such, based on previous research, it is necessary to evaluate the sensorial responses to acidity modulation, since climate change is predicted, less acidic wines and, therefore, it will be more and more frequent to acidify the wine, even in regions that previously these additions were totally unnecessary. Lactic acid was chosen based on work done last year (Ceciliani, 2017) when this acid was the most appreciated in the panel's assessment. The choice of succinic acid was due to two factors: a natural acid that forms during fermentation, is associated with a certain minerality (Baron & Fiala, 2012). However, it is described in the literature as sensorially uninteresting due to the salty and bitter taste (Coulter et al., 2004; Jackson, 2014) and OIV not does not allow its use. Succinic acid may be an alternative future acidifier. Therefore, the objectives of this study were:

i) To identify the Detection and Recognition Thresholds for tartaric, lactic and succinic acids.

ii) To understand the different levels of appreciation for lactic and succinic acids.

iii) To evaluate possible relations between the characterization of the tasters and their responses to the modulation of the acidity of the wine.

2. Material and methods

2.1 Tasting panel

Thirty-three Individuals (20 females and 13 males, between 19 and 40 years(average 24.7±5.3) were recruited from the student and faculty population of the University and from local community. In order to train the panel and perceive their different sensibilities several sessions were held where the different basic flavors were tested (sourness, sweetness, bitterness) and the mouthfeel sensation of astringency. All sessions took a place in the microbiology laboratory of ISA (Tapada da Ajuda, Lisbon).

2.2 Taster characterization

2.2.1 Questionnaires

Participants were asked to complete a brief questionnaire that collected basic demographic data (age, gender, nationality, education background). Their wine knowledge was obtained by endorsing the following items: *I don't drink wine; beginner; intermediate; very high*. The Vinotype was established through an online questionnaire (www.vinotype.com) based on the individual's wine preferences (Hanni, 2013) (Annex 1).

2.2.2 Saliva Status and Propylthiouracil phenotyping

Individuals were classified according to the Saliva flow, by following the procedure described by Smith et al. (1996) and with PROP status through the bitterness intensity of three PROP (Sigma, St. Louis, USA) solutions (0.032, 0.32, and 3.2 mM) presented in a increasing order of concentration. Individuals were initially trained in the use of the gLMS (generalized labeled magnitude scale) scale. The gLMS scale ranges from zero to 100, where zero = "no sense" and 100 = "strongest imaginable" (Bartoshuk, 2001).They were then asked to take the entire volume of each sample (20 ml) swirl it around for 10 s, and expectorate. After the sample was expectorated, they were asked to wait approximately 10 to 15 s and rate the maximum intensity they perceived on the gLMS provided. Participants were also asked to thoroughly rinse with filtered water between each sample.

Tasters were classified as non-tasters, tasters and super tasters based in the bitterness rating to the 0.32 mM PROP solution using the LMS Scale (Non-taster: ≤ 15.5; Taster: ≥ 15,5 and < 51; Super taster ≥ 51 (Tepper et al., 2001) (Annex 2).

2.2.3 Sucrose-liker/disliker classification

The Individuals were assessed regarding their sweet liking status: liking of sucrose solutions using visual analogue scales (VAS) to establish SL and SD classification The VAS (Visual analogue scale) scale used for the sweet liker test (15 cm) was marked with a neutral point at half scale length and had end-anchors from ‘‘Extremely unpleasant” to ‘‘Extremely pleasant” (Methven et al., 2016). In addition, the scale is divided into 3 equal segments by three marks (3.75 cm; 7.5 cm and 11.25 cm) (Annex 3).The procedure was the same as the PROP test.

2.3 Prototypical Taste Training

2.3.1 Wine

The wine used in this study is a Macabeu (un-oaked and low in flavor intensity) produced in the cellar of the ISA. Macabeu is a Spanish variety and was chosen for its neutral character. After the wine has been tasted, it was fixed acidity with calcium carbonate in order to make the adjustment of parameters, namely the acidity, more easily perceived by tasters. The basic physiochemical composition of the wine is given in Table 2.1.

Table 2.1 Physiochemical parameters of the base white wine.

Parameter	Value
pH	3.52
Ethanol (%vol)	11.3
Free SO2 (mg/l)	39
Total SO2 (mg/l)	105
Residual sugar (RS) (g/l)	0.7
Volatile acidity (g acetic acid /l)	0.23
Total acidity (g tartaric acid/l)	5.30

2.3.2 Procedure

All solutions used, were served/prepared with wine and stored at a temperature of 3-5 °C (in the refrigerator) for three days. Approximately two hours before serving, the solutions were brought to room temperature (18 ± 2 °C). Labeled 20 mL samples of tartaric acid (in concentrations of 0.15, 0.3, 0.6, 1.2, 2.4 g/L), sucrose (in concentrations of 1.5, 3, 6, 12 and 24 g/L) as well as Tannic acid (in concentrations of 0.093, 0.1875, 0.375, 0.75 and 1.5 g/L) were served to the participants.

Initially, they were asked to rinse their mouth with filtered water before starting the samples tasting. After, they were asked to holding the samples in their mouth for at least 10 seconds being sure that they cover all the mouth surfaces and wait for the sensation intensity to peak (10-15 seconds). Following, they were requested to draw a mark on the intensity scale (gVAS) as well as in the liking-one (VAS) according to their personal preference. The gVAS scale uses indicators as “no sensation” for the lowest/weakest scale end – point (0mm) and “strongest experienced sensation of any kind including pain” for the highest perceived end – point (100mm). The line was divided into quadrants (25, 50, and 75 mm, respectively) and broke up by three unlabeled mark points (Pickering and Kvas, 2016). The estimated break-point between the several samples, was approximately one minute (Anex 4). All the samples were presented blind, randomized and coded with three random digit numbers. During the tasting, two additional glasses were used, one served with the control wine, and one with spring water.

The main objective of this session was to evaluate the participants according to their ability in recognizing the perceived sensations each time (acidity, astringency, sweetness) as well the accomplishment of their overall training.

2.4 Sensory acidity evaluations

This study had two parts. The first part, threshold testing, was designed to estimate the detection and recognition thresholds of tartaric, lactic and succinic acids in white wine. The second study examined the preference of succinic and lactic acids in two white wines. A total of four test sessions were conducted on separate days.

2.4.1 Determination of sensory threshold

2.4.1.1 Wine

We looked for a wine that did not have a lot of acidity so that appreciation would be easier. We used a white bag in box from Tejo region “Sensato” (Table 2.2). This wine presents a neutral character.

Table 2.2. Wine used in determination of sensory thresholds.

Wine	Brand	Grape/Blend	Region
Sensato	Sensato 2016 Quinta das Casas Altas	Fernão Pires, Arinto and Moscatel	Tejo

2.4.1.2 Individuals

The thirty-three panelists from training session participated in this experiment.

2.4.1.3 Procedure

The determination of detection and recognition thresholds was done by the selected panel using 4 different concentrations of 3 acids (tartaric, lactic and succinic acid) added to “Sensato” base wine (table 2.3). Chosen concentrations were based on the work previously performed (Ceciliani, 2017). Table 2.4 shows the concentration of the different organic acids in relation to the equivalent concentration of tartaric acid. In all the sessions was used the triangular test. This test uses three samples to determine if an overall difference exists between two products (control wine and one that contains the substance under test). The samples were encoded with three-digit individual numbers (Annex 5). The order of presentation of concentrations was random. We asked the tasters to taste samples from left to right and to identify the different sample (detection threshold) and how does it feel/taste perceived (recognition threshold). The participants were instructed to that taste, spiting and drink water between each set of glasses. Was not given any information on wine so as not to influence the responses of the participants.

Table 2.3 Samples used in determination of sensory thresholds of organic acids.

Tartaric Acid	Lactic Acid	Succinic Acid
Control wine	Control wine	Control wine
0.40 g/l	0.48 g/l*	0.32 g/l*
0.80 g/l	0.96 g/l*	0.64 g/l*
1.6 g/l	1.92 g/l*	1.26 g/l*
3.3 g/l	3.84 g/l*	2.53 g/l*

* Expressed in tartaric acid

Table 2.4. Molecular weight (MW), protons per molecule, equivalent weight and multiplying factor to express the organic acid in tartaric acid concentration.

Acid	MW	Protons per molecule	Equivalent Weight	Multiplying Factor
Tartaric	150	2	75	1.00
Lactic	90	1	90	0.83
Succinic	118	2	59	1.27

2.4.2 Wine appreciation

2.4.2.1 Wines

The wines selected were ISA (A) and M.J.Freitas (B). The choice was due to their different fixed acidity. A was fresher and B as a less sour wine (Table 2.5).

Table 2.5. Wines used in wine appreciation.

Wine	Brand	Grape/Blend	Region
A	Encruzado 2016 ISA Cellar	Encruzado	Lisbon
B	M.J. Freitas 2016 Casa Ermelinda Freitas	Fernão Pires	Setúbal

2.4.2.2 Individuals

Only 26 panelists participated in this session and only these Individuals were included in subsequent data analysis.

2.4.2.3 Procedure

The solutions were served in INAO white glasses at room temperature, 20 ± 2 C°. Were given to tasters six glasses: Two of the glasses with the control wines and the other glasses with each of the base wines more 3 g/l of lactic acid and the other with 3 g/L of lactic acid and 0.32 g/L of succinic acid . The reason we use only these acids due to the fact that they are less-used acids and therefore, it was intended to study how they are appreciated. According to previous work, lactic acid was the most appreciated by the tasters as mentioned earlier (Ceciliani, 2017). We use only 0.32 g/L because the aroma is easily noticed from 0.64 g/L. The ratings have been applied on a VAS liking scale (15 cm) and, later, were measured (Annex 6)

2.5 Data analysis

Results obtained from panelists were subjected to variance analyses (α=0.05) with software R (www.r-project.org) to assess the influence of each segmentation type on wine liking. In order to evaluate possible interaction effects, we performed the factorial analyzes combining more than one segmentation type. For the segmentation types that showed influence on wine liking, mean comparisons were performed with Tukey's test (α=0.05).

3. Results and discussion

3.1 Taster characterization

The results of the taster characterisation are presented in Table 3.1 and individual responses are listed in Annex 7.

Table 3.1. Taster characterization according to the segments.

		Gender		Smoker			Vinotype			Wine expertise				Saliva flow		PTS			Sweet liking	
		F	M	Y	N	S	H	S	T	ND	B	I	VH	LF	HF	ST	T	NT	SL	SD
Gender	F	**20**	-	11	10	0	8	10	2	2	6	11	1	15	5	2	9	9	5	15
	M	-	**13**	2	7	4	4	8	1	1	4	8	0	5	8	0	10	3	6	7
Smoker	Y	11	2	**12**	-	-	8	4	0	1	5	5	1	8	4	0	7	5	4	8
	N	10	7	-	**17**	-	2	12	3	1	3	13	0	10	7	2	9	6	4	13
	S	0	4	-	-	**4**	2	2	0	1	2	1	0	2	2	0	3	1	3	1
Vinotype	H	8	4	8	2	2	**12**	-	-	3	5	4	0	8	4	1	4	7	6	6
	S	10	8	4	12	2	-	**18**	-	0	4	13	1	11	7	1	14	3	5	13
	T	2	1	0	3	0	-	-	**3**	0	1	2	0	1	2	**0**	1	2	0	3
Wine expertise	ND	2	1	1	1	1	3	0	0	**3**	-	-	-	2	1	1	1	1	1	2
	B	6	4	5	3	2	5	4	1	-	**10**	-	-	7	3	0	6	4	6	4
	I	11	8	5	13	1	4	13	2	-	-	**19**	-	11	8	1	11	7	4	15
	VH	1	0	1	0	0	0	1	0	-	-	-	**1**	0	1	0	1	0	0	1
Saliva flow	LF	15	5	8	10	2	8	11	1	2	7	11	0	**20**	-	1	11	8	7	13
	HF	5	8	4	7	2	4	7	2	1	3	8	1	-	**13**	1	10	4	4	9
PTS	ST	2	0	0	2	0	1	1	**0**	1	0	1	0	1	1	**2**	-	-	1	1
	T	9	10	7	9	3	4	14	1	1	6	11	1	11	10	-	**21**	-	4	15
	NT	9	3	5	6	1	7	3	2	1	4	7	0	8	4	-	-	**12**	6	6
Sweet liking	SL	5	6	4	4	3	6	5	0	1	6	4	0	7	4	1	4	6	**11**	-
	SD	15	7	8	13	1	6	13	3	2	4	15	1	13	9	1	15	6	-	**22**

Abbreviation: PTS, PROP Taster Status, F, Female, M, Male, ST, Supertaster, T, Taster, NT, Non-taster, LF, Low-flow, HF, High-flow, SL, Sweet liker, SD, Sweet disliker, Y, Yes, N, No, S, Sometimes, ND, No drink, B, Beginner, I, Intermediate, VH, Very high, H, Hipersensitive, S, Sensitive, T, Tolerant

Concerning the demographic questionnaire, the age, study background and country of origin were not used as segments because of the common features of the tasters, mostly Portuguese students of the master in Viticulture and Enology of ISA. Furthermore, none of the participants were not vegetarian or reported food allergies. Variable responses were obtained in the remaining self-reporting categories. Smoking habits yielded, out of the 33 tasters, 11 *smoker*s, 4 *occasional smokers* and 17 assumed themselves as *non-smokers*. The results of the Vinotype online questionnaire were 12 *Hypersentiv*e, 18 *Sensitive* and 3 *Tolerant*. In relation to wine consumption/expertise 3 *didn't drink wine*, 10 were *beginners*, 19 assumed to have a*intermediate knowledge* and one assumed to have *high knowledge* about wine.The physiological testes yielded 20 tasters as *high producers* of saliva(> 2.4 g/min) while the remaining 13 were *low producers* of saliva (< 2.4 g/min). Taste sensitivity to PROP separated into the expected 3 distinct classes, being 12 Non-tasters, 19 Tasters and 2 Supertasters. As regards to the sweet liking classification, 22 of them were evaluated as Sweetdislikers and 11 as Sweet likers.

3.2 Sensory thresholds of organic acids

3.2.1 Tartaric Acid

The concentrations detected as different from control or recognized as more acid are shown in table 3.2. These results allowed the calculation of the best estimate (BET) for the detection and recognition. The BET calculated for detection was of 1.65 g/L while the BET for recognition was of 3.24 g/l.

The detection threshold was also determined graphically as shown in figure 3.1. Considering 17 Individuals out of 21 as the minimum number in a triangular test to establish the difference (P=0.05). the interpolated value was 0.87 g/L. Recognition threshold could not be dermined by the graphical method because there is no minimum number agreeing judgements necessary to establish preference using α=0.05 for triangular comparison tests (n= 17).

Table 3.2 Individual and group best estimated threshold (BET) for the detection and recognition for **tartaric acid** (g/L). Correct choise indicated by 1 and incorrect by 0; highlighted grey cells indicate recognition of acid taste.

	Concentration (g/L)				Detection threshold		Recognition threshold	
Subject	0.4	0.8	1.6	3.2	BET	log(BET)	BET	log(BET)
1	0	0	1	0	4.53	0.66	4.53	0.66
2	0	0	1	0	4.53	0.66	4.53	0.66
3	0	1	0	1	2.26	0.35	2.26	0.35
4	1	0	0	1	2.26	0.35	4.53	0.66
5	0	0	0	1	2.26	0.35	4.53	0.66
6	0	1	1	0	4.53	0.66	4.53	0.66
7	0	1	1	1	0.56	-0.25	2.26	0.35
8	0	1	1	1	0.56	-0.25	4.53	0.66
9	0	1	1	1	0.56	-0.25	0.56	-0.25
10	0	1	0	1	2.26	0.35	4.53	0.66
11	0	1	1	0	4.53	0.66	4.53	0.66
12	0	1	1	0	4.53	0.66	4.53	0.66
13	1	0	1	0	4.53	0.66	4.53	0.66
14	1	1	1	1	0.28	-0.55	4.53	0.66
15	0	0	1	0	4.53	0.66	4.53	0.66
16	0	1	0	1	2.26	0.35	2.26	0.35
17	0	1	0	0	4.53	0.66	4.53	0.66
18	1	0	1	1	1.13	0.05	1.13	0.05
19	1	0	0	1	2.26	0.35	2.26	0.35
20	0	1	0	1	2.26	0.35	4.53	0.66
21	1	1	1	1	0.28	-0.55	4.53	0.66
22	0	0	1	1	1.13	0.05	4.53	0.66
23	0	0	1	1	1.13	0.35	4.53	0.66
24	0	0	0	0	4.53	0.66	4.53	0.66
25	1	0	1	1	1.13	0.05	2.26	0.35
26	0	1	1	1	0.56	-0.25	1.13	0.05
27	1	0	0	1	2.26	0.35	4.53	0.66
28	1	1	0	1	2.26	0.35	2.26	0.35
29	0	0	1	1	1.13	0.05	4.53	0.66
30	0	1	1	1	0.56	-0.25	4.53	0.66
31	1	1	1	1	0.28	-0.55	4.53	0.66
32	1	0	1	0	4.53	0.66	4.53	0.66
33	0	1	1	1	0.56	-0.25	0.56	-0.25
Correct answers	11	18	22	23	Mean log(BET)	0.216667	Mean log(BET)	0.511515
					Antilog (BET)	1.65	Antilog (BET)	3.24 g/L

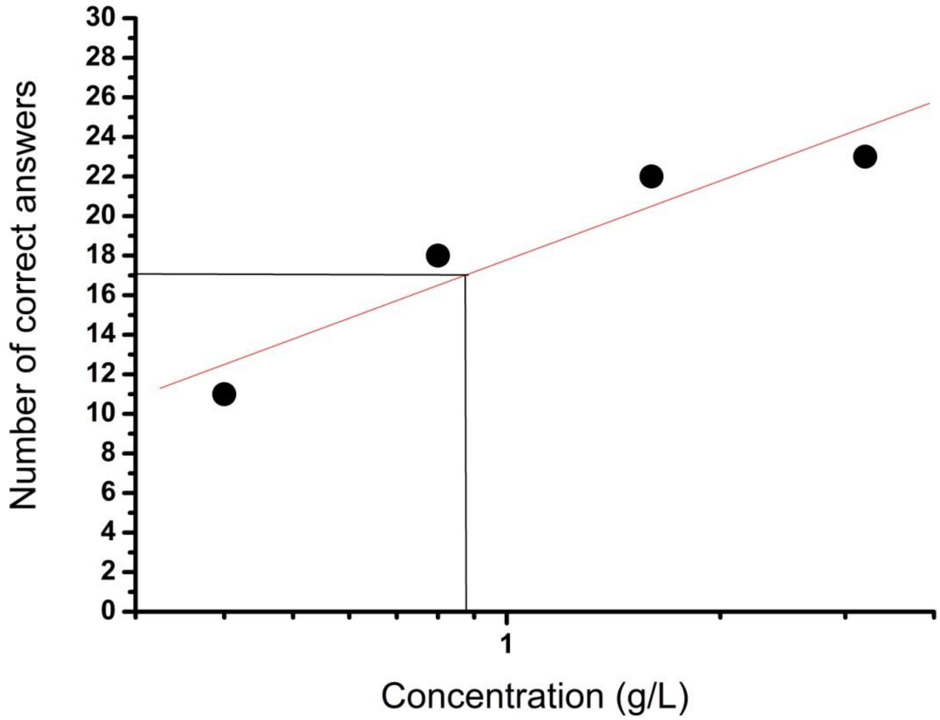

Fig. 3.1- Number of tasters (●) detecting differences in wine spiked with tartaric acid. Straight horizontal line (n = 17) represents minimum agreeing judgements necessary to establish preference using α=0.05 for triangular comparison tests (total number of tasters N=33). Straight line was obtained by linear correlation and vertical line indicates interpolation value.

3.2.2 Lactic Acid

The lactic acid concentrations detected as different from control or recognized as more acid are shown in table 3.2. These results allowed the calculation of the best estimate (BET) for the detection and recognition. The BET calculated for detection was of 1.98 g/L while the BET for recognition was of 3.48 g/L.

Table 3.3. Individual and group best estimated threshold (BET) for the detection and recognition for **lactic acid** (g/l). Correct choise indicated by 1 and incorrect by 0; highlighted grey cells indicate recognition of acid taste.

	Concentration (g/L)				Detection threshold		Recognition threshold	
Subject	0.48	0.96	1.92	3.84	BET	log(BET)	BET	log(BET)
1	0	0	0	1	2.72	0.43	2.72	0.43
2	0	1	0	0	5.43	0.73	5.43	0.73
3	1	1	1	1	0.34	-0.47	0.68	-0.17
4	1	1	0	1	2.72	0.43	5.43	0.73
5	1	1	0	1	2.72	0.43	5.43	0.73
6	0	1	0	1	2.72	0.43	5.43	0.73
7	0	0	1	0	5.43	0.73	5.43	0.73
8	1	0	1	1	1.36	0.13	2.72	0.43
9	1	1	0	1	2.71	0.43	5.43	0.73
10	0	0	0	0	5.43	0.73	5.43	0.73
11	1	0	1	1	1.36	0.13	1.36	0.13
12	1	1	1	1	0.34	-0.47	5.43	0.73
13	0	0	1	1	1.36	0.13	1.36	0.13
14	0	0	0	0	5.43	0.73	5.43	0.73
15	1	1	0	1	2.72	0.43	5.43	0.73
16	0	0	1	0	5.43	0.73	5.43	0.73
17	0	1	1	1	0.68	-0.17	0.68	-0.17
18	0	0	1	1	1.36	0.13	1.36	0.13
19	1	0	1	0	5.43	0.73	5.43	0.73
20	1	1	1	1	0.34	-0.47	5.43	0.73
21	1	0	1	0	5.43	0.73	5.43	0.73
22	0	0	0	1	2.72	0.43	5.43	0.73
23	1	0	0	1	2.72	0.43	5.43	0.73
24	0	0	1	1	1.36	0.13	5.43	0.73
25	1	1	0	1	2.72	0.43	5.43	0.73
26	0	0	0	1	2.71	0.43	5.43	0.73
27	0	1	1	1	0.68	-0.17	5.43	0.73
28	0	0	1	1	1.36	0.13	1.36	0.13
29	1	1	0	0	5.43	0.73	5.43	0.73
30	0	0	1	0	5.43	0.73	5.43	0.73
31	0	1	1	1	0.68	-0.17	0.68	-0.17
32	1	1	0	1	2.72	0.43	5.43	0.73
33	1	1	1	1	0.34	-0.47	1.36	0.13
Correct answers	16	16	18	24	Mean log(BET)	0.296182	Mean log(BET)	0.541837
					Antilog (BET)	1.98	Antilog (BET)	3.48 g/L

The detection threshold was also determined graphically as shown in figure 3.2. Considering 17 Individuals out of 33 as the minimum number in a triangular test to establish the difference (P=0.05), the interpolated value was 1.10 g/L. Recognition threshold could not be dermined by the graphical method because there is no minimum number agreeing judgements necessary to establish preference using α=0.05 for triangular comparison tests (n= 17).

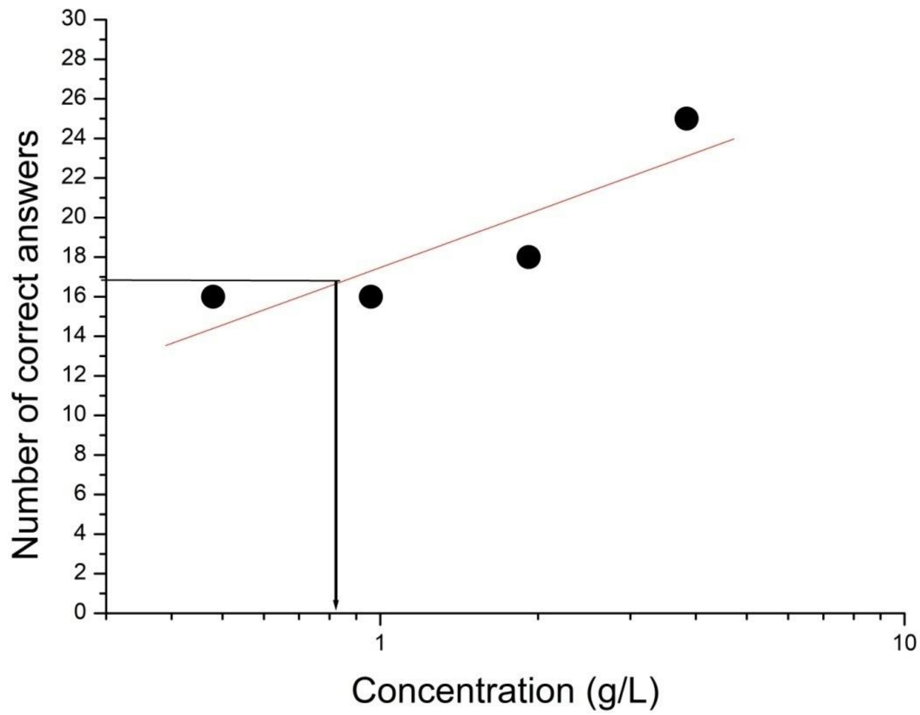

Fig. 3.2 -Number of tasters (●) detecting differences in wine spiked with lactic acid. Straight horizontal line (n = 17) represents minimum agreeing judgements necessary to establish preference using α=0.05 for triangular comparison tests (total number of tasters N=33). Straight line was obtained by linear correlation and vertical line indicates interpolation value.

3.2.3 Succinic Acid

The succinic acid concentrations detected as different from control or recognized as more acid are shown in table 3.4. These results allowed the calculation of the best estimate (BET) for the detection and recognition. The BET calculated for detection was of 0.88 g/L similar to the calculated detection threshold while the BET for recognition was of 1.05 g/L.

Table 3.4 Individual and group best estimated threshold (BET) for the detection and recognition for **succinic acid** (g/L). Correct choise indicated by 1 and incorrect by 0; highlighted grey cells indicate recognition of acid taste.

	Concentration (mg/L)				Detection threshold		Recognition threshold	
Subject	0.32	0.63	1.26	2.53	BET	log(BET)	BET	log(BET)
1	1	0	0	1	1.78	0.25	1.78	0.25
2	1	0	1	1	0.89	-0.05	0.89	-0.05
3	0	0	1	1	0.89	-0.05	0.89	-0.05
4	1	0	1	1	0.89	-0.05	0.89	-0.05
5	0	1	1	1	0.45	-0.35	0.45	-0.35
6	1	0	1	0	3.58	0.55	3.58	0.55
7	0	0	0	1	1.78	0.25	1.78	0.25
8	1	0	1	1	0.89	-0.05	0.89	-0.05
9	0	0	1	1	0.89	-0.05	0.89	-0.05
10	1	1	1	1	0.23	-0.64	0.23	-0.64
11	1	1	1	1	0.23	-0.64	0.23	-0.64
12	0	1	1	1	0.45	-0.35	1.78	0.26
13	0	0	1	1	0.89	-0.05	0.89	-0.05
14	1	1	1	1	0.23	-0.64	0.23	-0.64
15	1	1	1	0	3.58	0.55	3.58	0.55
16	0	1	0	0	3.58	0.55	3.58	0.55
17	0	1	1	1	0.45	-0.35	0.45	-0.35
18	0	0	0	1	1.78	0.25	1.78	0.25
19	0	0	1	1	0.89	-0.05	0.89	-0.05
20	1	1	0	0	3.58	0.55	3.58	0.55
21	1	1	1	1	0.23	-0.64	0.89	-0.05
22	0	0	0	1	1.78	0.25	1.78	0.25
23	0	1	1	1	0.45	-0.35	0.45	-0.35
24	0	0	0	1	1.78	0.25	1.78	0.25
25	0	0	0	1	1.78	0.25	1.78	0.25
26	0	0	1	1	0.89	-0.05	1.78	0.26
27	0	0	1	1	0.89	-0.05	0.89	-0.05
28	0	1	1	1	0.45	-0.35	0.45	-0.35
29	1	1	1	0	3.58	0.55	3.58	0.55
30	0	1	0	1	1.78	0.25	1.78	0.25
31	0	1	1	1	0.45	-0.35	0.45	-0.35
32	1	1	1	1	0.23	-0.64	3.58	0.55
33	1	1	1	1	0.23	-0.64	0.23	-0.64
Correct answers	14	17	24	28	Mean log(BET)	-0.05586	Mean log(BET)	0.02
					Antilog (BET)	0.879 g/l	Antilog (BET)	1.05 g/L

The detection threshold was also determined graphically as shown in figure 3.3. Considering 17 Individuals out of 33 as the minimum number in a triangular test to establish the difference (P=0.05), the interpolated value was 0.55 g/L, similar to the calculated detection threshold.

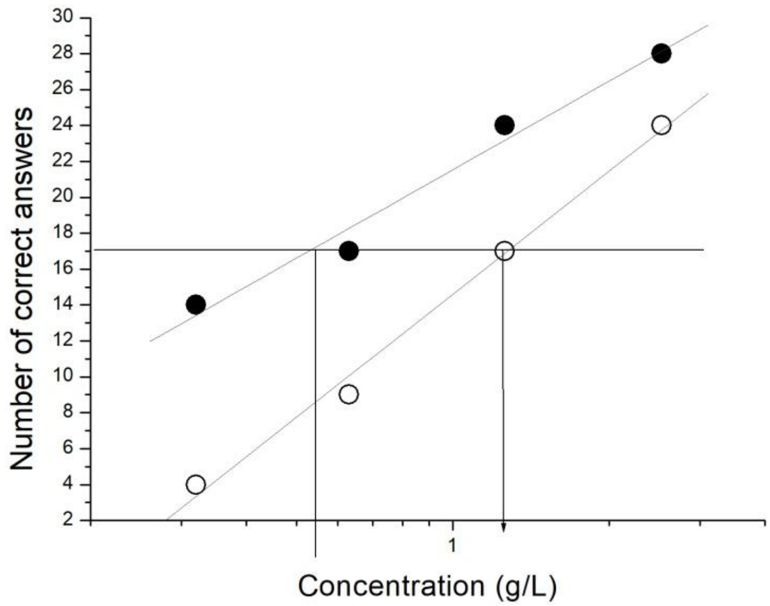

Fig. 3.3- Number of tasters detecting (●) and recognizing (○) differences in wine spiked with tartaric acid. Straight horizontal line (n = 17) represents minimum agreeing judgements necessary to establish preference using α=0.05 for triangular comparison tests (total number of tasters N=33). Straight line was obtained by linear correlation and vertical line indicates interpolation value.

3.2.4 Comparison among the thresholds of the organic acids

The overall detection responses to the acids are pooled in table 3.4 Tartaric and lactic acids induced similar responses while succinic acid seemed to be detected by a great number of individuals. Table 3.5 shows the frequency of citation of the taste and mouthfeel sensations elicited by the highest concentration of the organic acids used in the threshold determination.

Table 3.5 .Detection and Recognition Thresholds (g/L) for Tartaric, Lactic and Succinic Acids by both methods (BET) and geometrically method.

	Detection threshold		Recognition threshold	
	BET	Graphical method	BET	Graphical method
Tartaric Acid	1,64	0,87	3,24	ND
Lactic Acid	1,98	1,1	3,48	ND
Succinic Acid	0,88	0,55	1,05	0,78

Abbreviation: ND, Not determined.

Table 3.6. Frequence of citation of the taste and mouthfeel sensations elicited by the highest concentration of the organic acids used in the threshold determination.

Acid	Concentration (g/l)	Salty	Bitter	Sour	Sweet	Astrigent
Tartaric Acid	3,30	4	6	14	6	3
Lactic acid	3,84	3	6	21	2	1
Succinic acid	2,53	9	5	14	3	2

Determination of sensory threshold is an essential element in sensory analysis and is important for a variety of purposes including the study of ingredient variation limits in produts, in particular in winery. With regard to the frequency of citations of the taste and mouthfeel sensations (table 3.5), for the succinic acid, the second sensation more cited is salty, according to the expected. In our study, we obtained detection thresholds of about 1.64 g/L tartaric acid, roughly 0.3 g/L higher than the values previously obtained (Ceciliani, 2017). About lactic acid, the BET of 1.98 g/L, approximately 0.90 g/L more than obtained by the same author. As for succinic acid, some authors (Berg et al., 1955; Amerine et al., 1959), say the threshold in water around your 0.034-0.035 g/L (0.04 g/L expressed in tartaric acid), since wine is a complex matrix, succinic acid threshold should be lower, especially in salty or bitter wines. In our particular case, the succinic acid was more easily detected compared to the other two organic acids tested due to your scent easily noticed from 0.63 g/L. These values are high comparing with authors (above mentioned). However, Pickering & Rachel (2016) reported that with three samples to compare per set, higher levels of sensory fatigue are possible and/or greater cognitive loads, both of which can lead to higher thresholds. A decrease in the detection threshold due to taster familiarity has been seen in some studies (McBride and Laing 1979; Loryn et al., 2016). Another possibility can be considered, the prior training was short for untrained Individuals. Only 45% of tasters are students of Oenology compared with earlier studies made by our group (Ceciliani, 2017) 80% of the tasters are oenology students.

3.3. Effect of peritreshold concentrations of organic acids on wine liking

The liking scores given by the tasters to the control wine and to the wine spiked with different acids is shown in Figure 3.4 and in Table 3.7.

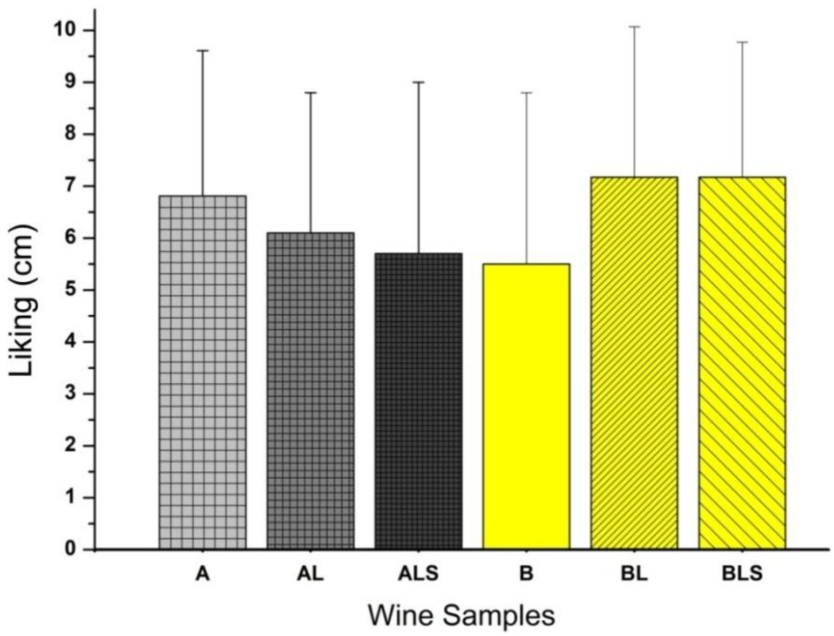

Fig. 3.4.-Liking scores of the white wine spiked with different concentrations of organic acids. A, ISA wine; AL, ISA wine spiked with lactic acid (1.92 g/L expressed in tartaric acid); ALS, ISA wine wine spiked with lactic acid (,92 g/l expressed in tartaric acid) and succinic acid (0.32 g/L expressed in tartaric acid); B, MJF wine; BL, MJF wine spiked with lactic acid (1,92 g/l expressed in tartaric acid), BLS, MJF wine spiked with lactic acid (1.92 g/L expressed in tartaric acid) and succinic acid (0.32 g/L expressed in tartaric acid).

Table 3.7 Mean liking scores (cm) of white wines spiked with organic acids.

Wines	Treatment	Mean	Std	Min	Max	Number of tasters
A	-	6.81	2.84	0.62	11.70	26
AL	Lactic acid	6.15	2.67	0.21	10.70	26
ALS	Lactic and succinic acids	5.71	3.30	0	12.00	26
B	-	6.03	2.98	0.80	11.80	26
BL	Lactic acid	6.93	2.75	0.60	12.80	26
BLS	Lactic and succinic acids	6.90	2.73	0.20	11.80	26

The wine as a factor did not influence the liking scores ($p = 0.513$). However, the tasters tended to give lower scores to wine when acidified, especially as regards ALS and give higher scores to acidified B wine. Between BL and BLS there seems to be no difference in liking scores. Wine A was was more acid and, therefore, acidification did not improve liking. The wine B, less sour, seemed to have benefited from acidification. This result shows that optimising acidification depends on the wine, despite the high variability of taster responses.

3.4 The influence of taster segmentation on liking scores

In order to understand the basis for the high variability of responses, the liking scores of all wines were firstly analyzed independently from the acidification treatments. An ANOVA was then performed in which liking scores for each wine were the dependent variables, and sex, wine expertise, sweet liking, smoker, vinotype and saliva production. Tukey's honest significant difference (HSD) was used as the means separation test throughout.

For analysis purposes, due to the low number of respondents in the Tolerant category (n=3), we collapsed the Tolerant and Sensitive categories to increase statistical power. The same was done for the smoking category. We collapsed the "Sometimes" and "Non-smokers" categories (n= 4).For the Wine Expertise and PROP Taster Status, we collapsed "I don't drink wine" (n= 3) with "Begginer" and Supertasters (n=2) with Tasters. The liking scores varied based on Vinotype, PROP Taster Status (PTS), Sweet liking, Smoker, Wine expertise (WE), but not Gender or Saliva flow (table 3.8).

Table 3.8. P-values from analysis of variance and HSD test applied to the liking of white wines and respective treatments (6 wines).

Categories	Class	P-values	Mean	Number of tasters
Gender	Male	0.178	6.79 a	11
	Female		6.16 a	15
Smoker	No	0.0035***	6.90 a	17
	Yes		5.51 b	9
Vinotype	Sensitive	0.0027***	6.96 a	16
	Hipersensitive		5.56 b	10
WE	Intermediate	0.0096 ***	6.89 a	16
	Begginer		5.67 b	10
Saliva flow	Low flow	0.554	6.54 a	15
	High flow		6.26 b	11
PTS	Taster	$1.34 \times 10^{-5***}$	7.26 a	11
	Non-Taster		5.28 b	15
Sweet liking	Sweet-liker	0.0096 ***	5.48 b	16
	Sweet disliker		6.84 a	10

Notes: *, **, ***, **** indicate significance at 0.05, 0.01, 0.001 and 0
Abbreviation: WE, Wine expertise, PTS, PROP Taster Status

In order to understand if the preferences vary with the wine style, we separated the liking scores for each wine. The results are shown in table 3.9.

Table 3.9. P-values from analysis of variance of wine liking scores for two wines and HSD test.

		Wines			
Factors	**Segments**	A		B	
		P-values	Mean	P-values	Mean
Gender	Male	0.6450	6.40 a	0.144	7.18 a
	Female		6.09 a		6.22 a
Smoker Status	No	0.228	6.51 a	0.0029***	7.30 a
	Yes		5.67 a		5.34 b
Vinotype	Sensitive	0.0701 *****	6.70 a	0.0152 ******	7.23 a
	Hipersensitive		5.46 a		5.65 b
Wine expertise	Intermediate	0.0495**	6.74 a	0.0944 *****	7.04 a
	Begginer		5.40 b		5.95 a
Saliva flow	Low flow	0.6990	6.33 a	0.6550	6.75 a
	High flow		6.07 a		6.45 a
PTS	Taster	0.0001****	7.27 a	0.0206 **	7.25 a
	Non-taster		4.79 b		5.77 b
Sweet liking	Sweet disliker	0.0242**	6.72 a	0.108	6.96 a
	Sweet liker		5.10 b		5.85 a

Notes: *, **, ***, **** indicate significance at 0.05, 0.01, 0.001 and 0
Abbreviation: WE, Wine expertise, PTS, PROP Taster Status

Regarding the gender factor, the preference did not vary was for both wines. The same can be said regarding the Saliva flow. In smoker category, the preference is different for less acidic wine (B). Smokers prefer this wine more when compared to non- smokers.

The Vinotype characterization could be related to wine appreciation. The individuals classified as “Sensitive” revealed that on average appreciate higher amount of acids when compared to “Hypersensitive”. Looking at wine expertise, the difference exists in more acidic wine (A), Individuals characterized as “Intermediate” prefer this style of wine than “Begginers”. The phenotype Prop has also influence on the apreciation, such as expected individuals classified as tasters have greater preference for tested wines in comparison to Non-Tasters. The sucrose-liker/disliker classification had influence on appreciation only in wine A, the participants classified as sweet dislike (SD) prefer this style comparing with sweet likers (SL).

3.4.1 Interaction among taster segments

An ANOVA in which liking scores for each wine were the dependent variables and all two-way interaction were independent variables was then completed. The significant two-way interactions for wine liking (considering 6 wines tested) are in table 3.9, for wine A in table 4 and in table 4.1 for the wine B.

Table 4. P-values from analysis of variance of the general wine liking scores.

	Gender	Smoker	Vinotype	WE	Saliva flow	PTS	SL
Gender	-	0.18.38	0.9501	0.5005	**0.0783***	**0.0012*****	**0.0017*****
Smoker	0.1838	-	0.1641	**0.0532****	0.4319	0.2428	**0.0823***
Vinotype	0.9501	0.1641	-	**0.0071****	**0.0039*****	**0.0456****	0.9865
WE	0.5005	**0.0532***	**0.0071****	-	**0.0262****	0.3610	0.4534
Saliva flow	**0.0783***	0.4319	**0.0039*****	**0.0262****	-	0.4250	0.3567
PTS	**0.0012*****	0.2428	**0.0456****	0.3610	0.4250	-	0.6491
SL	**0.0017*****	**0.0823***	0.9865	0.4534	0.3567	0.6491	-

Abbreviation: WE, Wine expertise, PTS, PROP Taster Status, SL, Sweet liking
Notes: *, **, ***, **** indicate significance at 0.05, 0.01, 0.001 and 0

As opposed to what we expected was not found interaction between Wine Expertise and PTS. According to Pickering & Hayes (2012) individuals may self-select for some professions or interests based on greater sensory acuity. The interaction between Gender and Sweet liking was expected according to Monneuse et al., 1991 preferences for sweet taste are known to vary as a function of sex .

Table 4.1 Significant interactions between factors on liking scores in wine A

	Wine A			
Factors	Class	P-values	Mean	Number of tasters
PTS x Gender	Taster Female	0.0014 ***	8.3 a	7
	Non-taster Male		6.4 ab	3
	Taster Male		6.4ab	8
	Non-taster Female		4.2 b	8
PTS x Sweet liking	Taster Sweet disliker	0.0706*	7.6 a	13
	Taster Sweet liker		5.3ab	2
	Non-taster Sweet liker		5.0 b	6
	Non-taster Sweet disliker		4.5 b	5
Vinotype x Salivaflow	Sensitive High-flow	0,0729 *	7.1 a	7
	Sensitive Low-flow		6.4 a	9
	Hipersensitive Low-flow		6.2a	6
	Hipersensitive High flow		4.4a	4
Vinotype x WE	Hipersensitive Intermediate	0,0856*	7.1 a	6
	Sensitive Beginner		6.9 a	4
	Sensitive Intermediate		6.6 a	12
	Hipersensitive Begginer		4.4 ab	4

Notes: *, **, ***, **** indicate significance at 0.05, 0.01, 0.001 and 0

In comparison with other tasters, female *Tasters* tended to rate wine A with higher scores. Male gave middle scores, regardless of being tasters or non-tasters. Female non-tasters are the ones who gave lower scores. preferences for sweet taste are known to vary as a function of sex (Monneuse et al, 1991). Whether a person was defined a sweet liker (SL) or disliker (SD) varied significantly with PTS (PROP Taster Status), with the majority of Tasters classified as Sweet dislikers. (13/15) The present results are in accordance with other studies (Yeomans e al., 2007), PROP tasters are more likely to be SD. SD tasters give wines higher scores when compares to the remaining tasters. SL tasters give middle scores and as for non-tasters (SL and SD) give the lowest scores. Hipersentive begginers seem to give lowers scores than the remaing segments compounds.

Table 4.2 Significant interactions between factors on liking scores in wine B.

Factors	Wine B			Number of tasters
	Classes	P-values	Mean	
Sweet liking x Gender	Sweet Liker Male	0.0048**	7.3 a	5
	Sweet disliker Male		7.0 a	6
	Sweet disliker Female		6.9a	12
	Sweet liker Female		3.3b	3
PTS x Vinotype	Taster Sensitive	0.0230*	7.8 a	12
	Non-taster Hipersensitive		5.9ab	7
	Non-taster Sensitive		5.5 ab	4
	Taster Hipersensitive		5.0 b	3
Saliva flow x Vinotype	High-Flow Sensitive	0.0238*	7.7 a	7
	Low-flow Sensitive		6.9a	9
	Low-flow Hipersensitive		6.5 ab	6
	High-flow Hipersensitive		4.4 b	4
Saliva flow x Smoker Status	High flow Yes	0.0856.	8.6 a	4
	Low-Flow Yes		7.5 a	6
	Low-Flow No		6.2 ab	9
	High- Flow No		5.2 b	7
Saliva Flow x Wine expertise	High-Flow Intermediate	0.0151*	7.5 a	7
	Low-flow Beginner		6.9 ab	6
	Low-flow Intermediate		6.7 ab	9
	High-flow Beginner		4.6 b	4

Notes: *, **, ***, **** indicate significance at 0.05, 0.01, 0.001 and 0

Female SL tended to give lower scores than the other classes (female SD, male SL or male SL). Tasters-sensitive gave higher scores, non-tasters seem to score less followed by the hypersensitive tasters. Hipersensitive HF gave lower scores to wine B unlike sensitive high-flow who rated it with higher scores. LF assumes a middle position. Smokers seem to prefer wine B whereas non-Smokers HF showed not to like it as much.

3.4.2 Effect of acidification on wine liking according to taster segmentation

The effect of acidification on wine liking was not demonstrated when all tasters' responses were analyzed, as shown previously in Figure 3.4 However, a tendency to decreasing liking was observed in the sourer wine (A) in the opposite direction of the less acid wine (B). Therefore, we decided to evaluate the effect of acidification separating the hedonic scores according to each taster category to assess if any category could be sensitive to wine acidification treatments. The results are illustrated in Figure 3.5 and the statistical treatment results in Tables 4.3 to 4.5.´

Regarding to wine A, the intermediate and beginners categories decreasing their preference with acidification. While in wine B, the preference of these tasters increasing with acification, the beginners seems to prefer the wine with lactic and succinic acids and intermediate prefers wine with lactic acid. In vinotype, Sensitive and hypersensitive Individuals responds negatively to wine acidification in wine A, in wine B, there is a tendency to sensitive prefer the lactic adition. In category of Prop, we observed that tasters, in wine A, rated all wines similary comparing with non-tasters that decreasing liking with acidification. In wine B, both categories consider that wine has improved with acidification. Sweet dislikers preferred all wines, in opposition, as expected, sweet likers decreasing your preference with acidification. In wine B, sweet dislikers rated all the wines similary and sweet likers consider that wine improve with acidification.

When analyzed control and wines with lactic acid (Table 4.3), we observed both wines were better scored by intermediate and sensitive tasters comparing with beginners and hipersentive, respectively. PROP Tasters rated higher wine A comparing with non-tasters.

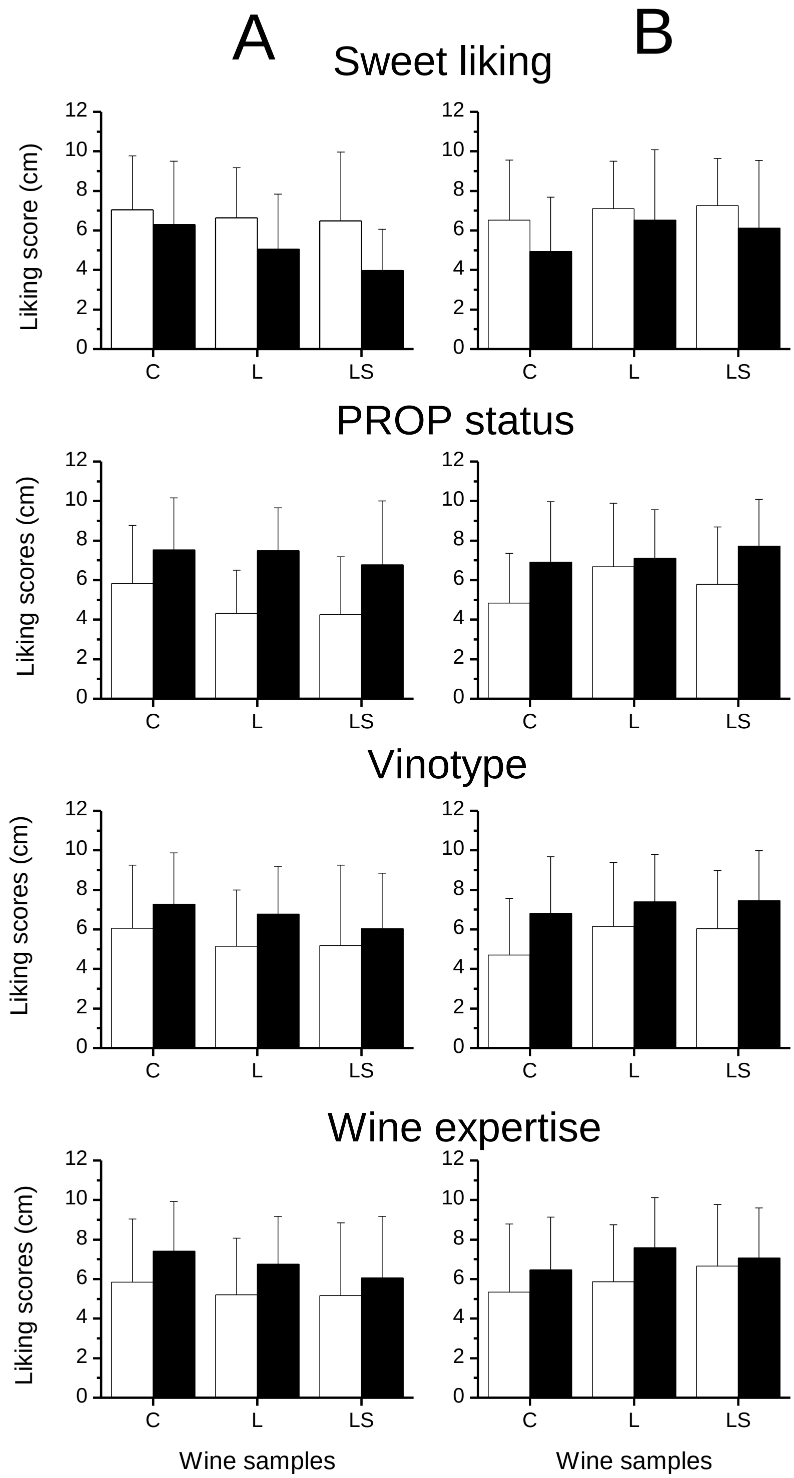

Fig. 3.5- Effect of acidification (C, control; L, lactic acid addition; LS, Lactic and succinic acids addition) on liking scores in wine A and B according to taster category (white bars: sweet dislikers, PROP Non-tasters, hypersensitive, begginers; black bars: sweet likers, PROP tasters, sensitive, intermediates). Horizontal bars indicate standard deviation.

Table 4.3. The influence of different segments on the liking scores in a control and wine spiked with lactic acid.

Factors		A		B		Number of tasters
	Segments	P-values	HSD Test	P-values	HSD Test	
Vinotype	Sensitive Hipersensitive	0.0677 *	7.03 a 5.60 a	0.0435 **	7.11 a 5.47 b	15 11
Wine expertise	Intermediate Beginner	0.0447 **	7.08 a 5.52 b	0.0801*	7.03 a 5.60 a	16 10
PROP Taster Status	Taster Non-taster	0.0010 ****	7.51 a 5.07 b	0.1220	-	15 11
Smoker	Non-smoker Smoker	0.2070	-	0.0533*	7.04 a 5.42 a	17 9

Notes: *, **, ***, **** indicate significance at 0.05, 0.01, 0.001 and 0

When analyzed control and LS wines (Table 4.4), tasters preferred these wines comparing with non-tasters. Smokers rated higher wine B and BLS than non-smokers.

Table 4.4. The influence of different segments on the liking scores in a control and wine spiked with lactic and succinic acids.

Factors		A		B		Number of tasters
	Segments	P-values	Mean	P-values	Mean	
PROP Taster Status	Taster Non-taster	0.0135	7.15 a 5.03 b	0.0511 *	6.78 a 4.25 a	15 11
Sweet liking	Sweet disliker Sweet liker	0.0784*	6.76 a 5.12 a	0.0699 *	6.49 a 3.96 a	16 10
Smoker	Non-smoker Smoker	0.6090	-	0.0160**	7.16 a 5.18 b	17 9

Notes: *, **, ***, **** indicate significance at 0.05, 0.01, 0.001 and 0.

The comparison between segments in acidified wines is shown in Table 4.5, revealing that acidification treatment was appreciated differently by smokers and sensitives in wine B. Sweet dislikers and PROP Tasters preferred the acidified wines (AL and ALS).

Table 4.5 Comparison between the two acidification treatments.

Factors		A		B		Number of tasters
	Segments	P-values	HSD Test	P-values	HSD Test	
Smoker	No Yes	0.2240	-	0.003 ***	7.70 a 5.43 b	17 9
Vinotype	Sensitive Hipersensitive	0.1410	-	0.0837 *	7.43 a 6.09 b	15 11
Sweet liking	Sweet disliker Sweet liker	0.0202 **	6.56 a 4.51 b	0.289	-	16 10
PROP Taster Status	Taster Non-taster	0.0003 ****	7.14 a 4.28 b	0.118	-	16 10

Notes: *, **, ***, **** indicate significance at 0.05, 0.01, 0.001 and 0.

Overall, these results show that there are tasters in certain segments that recognize the influence of acidification on wine liking. For instance, Sensitive, Sweet dislikers and PROP Tasters yielded higher liking scores for most of the acidified wines than Hypersensitive, sweet likers and PROP Non-Tasters.

4. Conclusions

In this work we evaluated the sensory responses given by a trained panel to changes in wine acidity. Tartaric and lactic acids induced similar responses while succinic acid displayed lower sensory thresholds, mainly because of its smell, easily detected above 0.63 g/L (expressed in tartaric acid). The main limitation of these determinations was the relatively small number of samples on which the threshold estimates were based. In addition the threshold values were higher compared with those previously obtained by Ceciliani (2016), probably because we used a trained panel with less experience in the recognition of acidity.

The effect of peritreshold concentrations on wine appreciation was similar for the 6 tasted wines. However, the tasters tended to give lower scores to the wine with higher acidity after acidification, while acidification tended to be more appreciated in the less sour wine. These results demonstrate the influence of the wine characteristics on the outcome of the acidification process. In addition, taster segmentation revealed that some categories were able to recognize the improving effect of acidification on wine liking while others responded negatively to acidification. This different evaluation was very complex, depending on taster category, wine style and type of acidulant.

These sensory studies are very complex, the taste sensitivity and preference variation of individuals is very large. Therefore, it is recommended in the future to use a higher number of tasters in order to obtain more robust conclusions.

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Annexes

Annex 1

Date___/__/______

Name____ Age_________

Gender (F/M) ____ Country ___________________ Study Background _______________________

Smoker (Y/N/Sometimes)_____Vegetarian (Y/N)_____Food Allergy (Y/N) _____

Vinotype _______________________

Wine Expertise

I don't drink wine	
Beginner	
Intermediate	
Very high	

Taste the sample given to you, hold it in the mouth for 10/15 seconds. Spit it out. Hold for another 10 seconds and spit in the plastic cup for a minute.

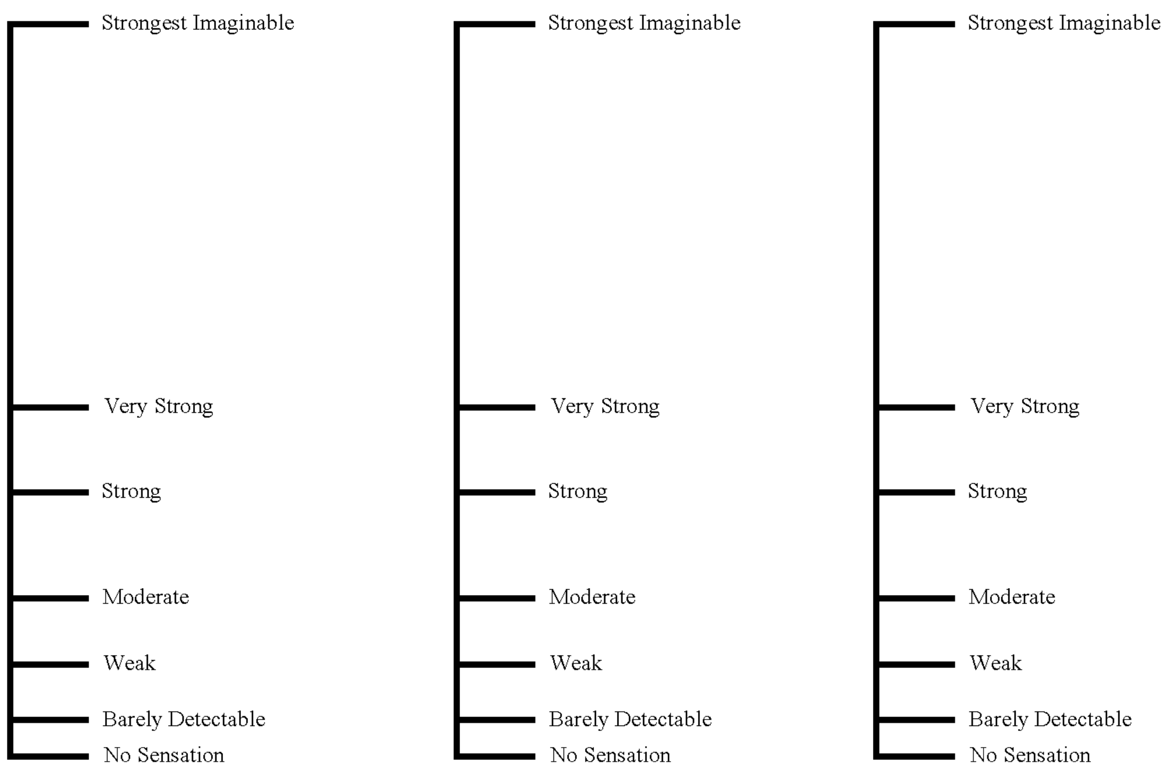
Initial Weight	
Total Weight	
Saliva Weight	

Annex 2

Date___/__/______

Name ____

Put the sample in the mouth, rinse for 10 seconds, being sure that you cover all the mouth surfaces and wait for the bitterness intensity to peak (10-15s). The maximum intensity is 10 seconds after spiting. After you taste the first sample rate the intensity of the sensation by drawing a mark on the LMS Scale. Rinse with spring water and wait 1 minute in between samples. Repeat the same procedure with the other 2 samples.



Annex 3

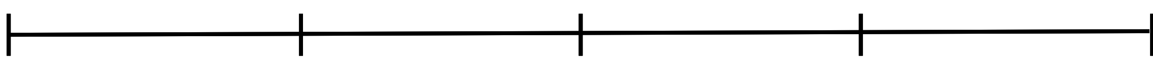
Name____

Put the separate sample in the mouth, rinse for 10 seconds, being sure that you cover all the mouth surfaces and wait for the sensation to peak (1—15s.) The maximum intensity is 10 seconds after spiting. After, draw a mark on the liking scale according to your personal preference.

Liking

Dislike extremely

Like extremely



Annex 4

Date___/__/______

Name____

Please taste each triangle set and try to identify which one of the three glasses has the different content (two of the three glasses in each set are completely similar). Write down the different sensation/taste that you receive. Note that even if you are not able to identify the different glass, you should choose one in any case. Please try to complete each triangle set in one repetition, and avoid coming back, as it might lead you to confusions.

1

987
398 409

2

941
265 357

3

327
982 640

4

327
982 640

	N° of the different glass	**Different perceived taste**
1		
2		
3		
4		

Annex 5

Name____

Rinse with water before beginning. Put the sample in the mouth, rinse for 10 seconds, being sure that you cover all the mouth surfaces and wait for the sensation intensity to peak (10-15s). The maximum intensity is 10 seconds after spiting. After you taste the first sample rate the intensity of the sensation by drawing a mark on the intensity scale. After, draw a mark on the liking scale according to your personal preference. Rinse with spring water and wait 1 minute between samples. Repeat the same procedure with the other samples.

432.

Intensity

No sensation

Strongest experienced sensation
of any kind including pain

Liking

Dislike extremely

Like extremely

365.

No sensation

Intensity

Strongest experienced sensation
of any kind including pain

Liking

Dislike extremely

Like extremely

892.

Intensity

No sensation

Strongest experienced sensation of any kind including pain

Liking

Dislike extremely

Like extremely

213.

Intensity

No sensation

Strongest experienced sensation of any kind including pain

Liking

Dislike extremely

Like extremely

501.

Intensity

No sensation

Strongest experienced sensation of any kind including pain

Liking

Dislike extremely

Like extremely

Annex 6

Date___/__/______

Name____

Taste each of the wines separately, after spitting, draw a mark on the liking scale according to your personal preference.

756

Dislike extremely — Like extremely

567

Dislike extremely — Like extremely

657

Dislike extremely — Like extremely

834

Dislike extremely — Like extremely

438

Dislike extremely — Like extremely

384

Dislike extremely — Like extremely

Annex 7

Table 1. PHysiochemical parameters of the Sensato wine

Wine	Density (g/ml)	pH	Alcohol strength (% vol)	Free SO2 (mg/l)	Total SO2 (mg/l)	Volatile acidity (g acetic acid./l)	Total acidity (g tart. acid/l)	Residual Sugar (g/L)
Sensato (Control wine)	0.9912	3.56	12.4	4	50	0.27	4.95	0.58
Sensato + 0.48 g/L Lactic	0.9912	3.48	12.4	4	50	0.27	5.55	0.58
Sensato + 0.96 g/L Lactic	0.9912	3.43	12.4	4	50	0.27	5.7	0.58
Sensato + 1.92 g/L Lactic	0.9912	3.37	12.4	4	50	0.27	6.3	0.58
Sensato + 3.84 g/L lactic	0.9912	3.27	12.4	4	50	0.27	7.5	0.58
Sensato + 0.4 g/L Tartaric	0.9912	3.43	12.4	4	50	0.27	5.4	0.58
Sensato + 0.8 g/L Tartaric	0.9912	3.40	12.4	4	50	0.27	6	0.58
Sensato + 1.6 g/L Tartaric	0.9912	3.33	12.4	4	50	0.27	6.9	0.58
Sensato + 3.2 g/L Tartaric	0.9912	3.18	12.4	4	50	0.27	8.25	0.58
Sensato + 0.32 g/L Succinic	0.9912	3.52	12.4	4	50	0.27	5.4	0.58
Sensato + 0.63 g/L Succinic	0.9912	3.51	12.4	4	50	0.27	6.15	0.58
Sensato + 1.26 g/L Succinic	0.9912	3.49	12.4	4	50	0.27	6.75	0.58
Sensato + 2.53 g/L Succinic	0.9912	3.45	12.4	4	50	0.27	8.25	0.58

Table 2. Physiochemical parameters of the wines used in wine appreciation

Wine	Density (g/ml)	pH	Alcohol strength (% vol)	Free SO2 (mg/l)	Total SO2 (mg/l)	Volatile acidity (g acetic acid./l)	Total acidity (g tart. acid/l)	Residual Sugar (g/L)
A	0.9906	3.32	12.1	27	95	0.17	6.00	0.91
AL	0.990.6	3.17	12.1	27	95	0.17	7.65	0.91
ALS	0.9906	3.16	12.1	27	95	0.17	7.95	0.91
B	0.9902	3.61	12.2	45	88	0.27	4.35	1.74
BL	0.9902	3.42	12.2	45	88	0.27	6.00	1.74
BLS	0.9902	3.43	12.2	45	88	0.27	6.15	1.74

Annex 7

Name	Taster	Age	Gender	Country	Smoker	Vinotype	PTS	Smoker	Saliva Status	W.E	Sweet liking	Study background	Vegetarian	Food Allergy
Ana Rita Casquinha	1	24	F	PT	Y	H	NT	Y	LF	I	SD	Biochemistry	N	N
Ana Sofia Caldeira	2	23	F	PT	Y	S	T	Y	LF	I	SD	Agronomic Engineer	N	N
Ana Sofia Domingos	3	21	F	PT	N	S	NT	N	LF	I	SL	Agronomic Engineer	N	N
Ana Teresa Araújo	4	22	F	PT	N	T	NT	N	LF	I	SD	Biochemistry	N	N
António Lourenço	5	22	F	PT	N	S	T	N	LF	I	SD	Food Engineer	N	N
Bruno Alencar	6	30	M	BR	N	T	T	N	HF	B	SD	Internacional relations	N	N
Bruno Moreira	7	22	M	PT	S	H	NT	S	HF	I	SL	Food Engineer	N	N
Catarina Leal	8	20	F	PT	Y	H	T	Y	LF	I	SD	Biology	N	N
Chynthia Vieira	9	38	F	BR	N	S	NT	N	LF	I	SD	Law	N	N
Daniela Miguel	10	24	M	PT	N	S	T	N	LF	I	SD	Agronomic Engineer	N	N
Eva Christ	11	26	F	G	Y	S	T	Y	HF	VH	SD	Wine Business	N	N
Filipe Orvalho	12	23	M	PT	N	S	NT	N	LF	I	SL	Gestion	N	N
Francisco Coelho	13	23	M	PT	S	H	T	S	HF	ND	SD	High school	N	N
Guilherme Maia	14	23	M	PT	Y	H	T	Y	LF	I	SD	Agronomic Engineer	N	N
Henrique Duarte	15	23	M	PT	S	S	T	S	LF	B	SL	Envirnonment Engineer	N	N
Inês Barroso	16	22	F	PT	N	H	ST	N	LF	ND	SL	Biology	N	N
Joana Borrões	17	22	F	PT	Y	S	T	Y	HF	I	SD	Agronomic Engineer	N	N
João Costa	18	23	M	PT	N	S	T	N	HF	I	SD	Organic farming	N	N
João Maria	19	23	M	PT	N	S	T	N	HF	I	SD	Agronomic Engineer	N	N
José Côrrea	20	24	M	PT	N	S	T	N	HF	I	SD	Agronomic Engineer	N	N
Leonel Covas	21	36	M	PT	N	S	T	N	HF	I	SL	Agronomic Engineer	N	N
Maria Silva	22	19	F	PT	N	S	T	N	LF	B	SD	Food Engineer	N	N
Marta Frade	23	23	F	PT	Y	H	NT	Y	HF	B	SL	Architecture	N	N
Marta Gonçalves	24	24	F	PT	Y	S	T	Y	LF	B	SD	Food Engineer	N	N
Marta Vendeiro	25	37	F	PT	N	T	NT	N	HF	I	SD	Agronomic Engineer	N	N
Miguel Zilhão	26	23	M	PT	Y	H	NT	Y	HF	B	SL	Civil Engineering	N	N
Mónica Ramalhal	27	22	F	PT	Y	H	T	Y	LF	B	SL	Biology	N	N
Natacha Maganete	28	40	F	PT	N	S	T	N	LF	I	SD	Pharmaceutical Sciences	N	N
Pedro Soares	29	23	M	PT	S	S	T	S	LF	B	SL	Architecture	N	N
Sara Leal	30	23	F	PT	Y	H	NT	Y	LF	ND	SD	Architecture	N	N
Susana Trafaria	31	24	F	PT	N	S	ST	N	HF	I	SD	Agronomic Engineer	N	Y
Vera Maia	32	24	F	PT	N	H	NT	N	LF	B	SD	Biology	N	N
Vitória Pais	33	20	F	PT	Y	H	NT	Y	LF	B	SL	Agronomic Engineer	N	N